Structure-Based Design, Synthesis, and Biological Evaluation of Irreversible Human Rhinovirus 3C Protease Inhibitors. 6. Structure−Activity Studies of Orally Bioavailable, 2-Pyridone-Containing Peptidomimetics

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The structure-based design, chemical synthesis, and biological evaluation of various 2-pyridone-containing human rhinovirus (HRV) 3C protease (3CP) inhibitors are described. These compounds are comprised of a peptidomimetic binding determinant and a Michael acceptor moiety, which forms an irreversible covalent adduct with the active site cysteine residue of the 3C enzyme. The 2-pyridone-containing inhibitors typically display improved 3CP inhibition properties relative to related peptide-derived molecules along with more favorable antiviral properties. The cocrystal structure of one pyridone-derived 3CP inhibitor complexed with HRV-2 3CP is also described along with certain ab initio conformation analyses. Optimization of the 2-pyridone-containing compounds is shown to provide several highly active 3CP inhibitors (k_{obs}/[I] > 500 000 M^{-1} s^{-1}) that function as potent antirhinoviral agents (EC_{50} = <0.05 μM) against multiple virus serotypes in cell culture. One 2-pyridone-containing 3CP inhibitor is shown to be bioavailable in the dog after oral dosing (F = 48%).

Introduction

The human rhinoviruses (HRVs) are members of the picornavirus family and are the single most significant cause of the common cold in man.[1−3] Although considerable effort has been expended in the past to identify clinically efficacious antirhinoviral agents, to date, there are no marketed antivirals available for the treatment of this prevalent human pathogen. In addition, the large number of known HRV serotypes (>100) makes the development of vaccines seem unlikely.[4] Previous attempts at antirhinoviral therapeutic identification include the use of interferon,[2a,c,e] the disruption of virus−receptor (ICAM-1) interactions,[5] the examination of capsid-binding antipicornaviral compounds,[6] and the use of other miscellaneous agents.[7,8] In contrast, the antirhinoviral development efforts at Agouron have focused on disruption of an early, essential step in the HRV replication cycle that involves the proteolytic processing of the large polypeptide produced by cellular translation of the positive strand viral RNA genome. The majority of this processing is effected by the HRV 3C protease (3CP),[9,10] a cysteine protease possessing minimal homology with known mammalian enzymes but which structurally resembles members of the trypsin protein family.[10,11] Because of its importance in the viral replication cycle and the expected high conservation of its active site residues among all known HRV serotypes,[12] it is believed that potent and selective 3CP inhibitors might function as broad-spectrum antirhinoviral therapeutic agents. Indeed, 3CP has been the target of several research programs that sought to identify such antirhinoviral compounds,[13] although only one 3CP inhibitor has currently advanced to the stage of human clinical trials (see below).

Previously, we described the design and development of substrate-derived[9,14] peptidyl and peptidomimetic 3CP inhibitors that incorporate C-terminal Michael acceptor moieties (e.g., compound **1**, Figure 1).[15−17] These compounds irreversibly inhibit 3CP by forming a covalent adduct with the active site cysteine residue of the enzyme and exhibit broad-spectrum antirhinoviral activity in cell culture assays. Our earlier studies culminated with the selection of one particularly active peptidomimetic compound, ruprintrivir (USAN, AG7088), for clinical development as a nasally delivered antirhinoviral agent.[18−20] This molecule recently demonstrated encouraging activity in human clinical trials in which healthy volunteers were deliberately exposed to several HRVs and is currently the subject of large-scale phase II studies to examine its effectiveness against naturally acquired colds.[21] Concurrent with these advances, we also wanted to examine orally bioavailable 3CP inhibitors for potential treatment of the common cold and other picornaviral infections.[22] Unfortunately, potent 3CP inhibitors with sufficient oral bioavailability to warrant further development were not encountered in the pool of peptide and peptidomimetic compounds that led to ruprintrivir (AG7088).[23] Therefore, we continued to seek additional potent, 3CP inhibitors with nonpeptidic chemical structures that were distinct from the molecules that comprised our earlier studies. It was

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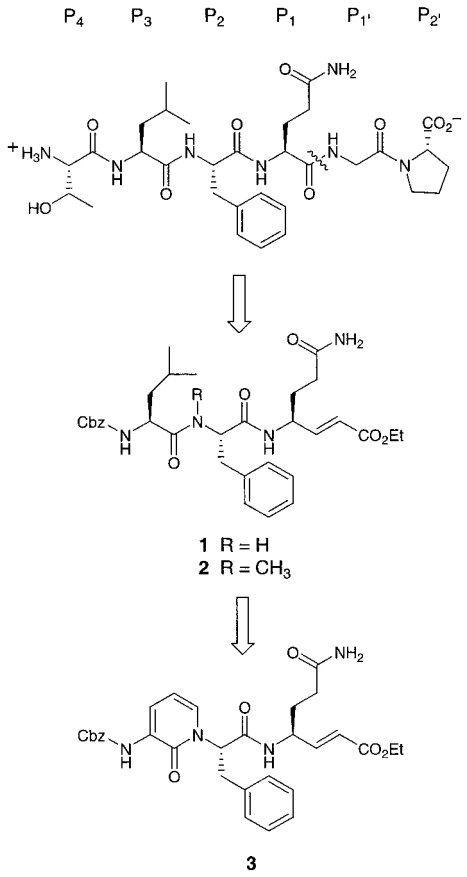
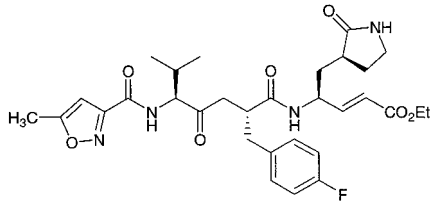


Figure 1. Design of 2-pyridone-containing HRV 3CP inhibitors.

envisioned that such compounds would aid in the identification of orally bioavailable 3CP inhibitors by further diversifying the physical and biological properties (e.g., mw, mp, water solubility, metabolic stability, etc.) of our existing anti-3CP agents. The discovery and development of one such series of potent, orally bioavailable, 3CP inhibitors are described below.



Ruprintrivir (AG7088)
$k_{obs}/[I]$ = 1,470,000 $M^{-1}s^{-1}$
EC_{50} = 0.013 μM
(HRV-14)

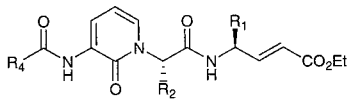
Inhibitor Design and Initial Structure−Activity Studies

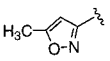
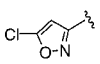
Analysis[14] of the HRV-2 3CP-**1** X-ray crystal structure[15] suggested that the P_3 amino acid residue contained in the inhibitor **1** could be replaced with a 3-amino-2-pyridone moiety without significantly affecting key protein−ligand interactions (e.g., compound **3**, Figure 1). Such replacement seemed plausible since (i) the P_3 side chain of **1** did not appreciably contact the 3CP protein, (ii) the P_2−P_3 amide NH of **1** did not contribute significantly to 3CP recognition and could be substituted with a ketomethylene[24] or an *N*-methyl moiety[1] (e.g., compound **2**, Figure 1), (iii) the β-sheet hydrogen-bonding interactions observed between the P_3 residue of **1** and the 3CP would be maintained, and (iv) the previous utilization of related 2-pyridones afforded potent inhibitors of other trypsinlike proteases that are structurally similar to 3CP.[25,26] The above crystal structure analysis also suggested that in contrast to most previous applications of 2-pyridone-containing enzyme inhibitors, access to the 3CP S_2 binding pocket would best be achieved from a position adjacent to the P_2 amide carbonyl moiety and not from the pyridone ring itself. In the event, incorporation of an appropriate P_3 3-amino-2-pyridone moiety into the inhibitor design afforded a compound (**3**), which displayed greatly improved anti-3CP and antiviral activity relative to the related peptidyl molecule **1** (Table 1).[27] Although the precise nature of these improvements could not be identified with certainty,[28] they confirmed the above inhibitor design hypothesis and prompted an extensive structure−activity study of 2-pyridone-containing 3CP inhibitors.

Initial 2-pyridone modifications involved variation of the P_2 benzyl moiety of **3** through the introduction of functional groups that were guided by previous studies of tripeptidyl 3CP inhibitors.[29] Thus, a compound containing a 4-fluorobenzyl P_2 substituent (**4**) displayed anti-3CP and antiviral activities nearly equal to that of **3**, while a molecule that incorporated a 3,4-difluorobenzyl moiety (**5**) exhibited significantly improved inhibition of both the 3C enzyme and the rhinovirus virulence in cell culture (Table 1). The antiviral activity trends of compounds **4** and **5** were also observed with two HRV serotypes other than type 14, suggesting that they might apply to a large number of rhinoviruses (Table 1). Somewhat surprisingly, and in contrast to our earlier studies of tripeptidyl molecules, saturation of the P_2 benzyl moiety resulted in a drastic loss of both 3CP inhibition activity and antirhinoviral properties (compound **6**, Table 1). The origins of the activity reductions observed with compound **6** have not been rigorously determined but may result from an unfavorable solution conformation of the unbound ligand. Importantly, a 2-pyridone-containing compound that lacked the P_2 substituent entirely (**7**) was an extremely poor 3CP inhibitor and antiviral agent (Table 1). These results suggested that 2-pyridone 3CP inhibitors required some type of P_2 moiety for effective 3CP recognition but did not establish a minimum size for such a substituent.

Having completed the brief exploration of P_2 structure−activity relationships in the 2-pyridone series of 3CP inhibitors described above, we next examined modification of the *N*-terminal (P_4) substituent. Substitution of the Cbz moiety present in **3** with a simple acetyl group resulted in drastic loss of both 3CP inhibitory properties and antirhinoviral activity (compare **8** with **3**, Table 1). Both of these activities were regained somewhat by incorporation of a P_4 amide derived from cyclopentanecarboxylic acid into the inhibitor design (compound **9**, Table 1). Interestingly, introduction of a 1,3-dithiolane-2-carboxylic acid-derived *N*-terminal amide afforded high levels of 3CP inhibition and antiviral activity when the resulting compound (**10**) was tested against HRV serotype 14. Unfortunately, the molecule

Table 1. 2-Pyridone-Containing 3CP Inhibitors



Compd No.	R_1	R_2	R_4	Prep.[a]	Formula[b]	serotype	k_{obs}/[I] ($M^{-1}s^{-1}$)[c]	EC_{50} (μM)[d]	CC_{50} (μM)[e]
1		(see Figure 1)		ref. 15	$C_{32}H_{42}N_4O_7$	14	25,000	0.54	>320
3	$CH_2CH_2CONH_2$	CH_2Ph	$PhCH_2O$	A	$C_{31}H_{34}N_4O_7 \cdot 0.25H_2O$	14	114,000	0.033	50
						1A	ND	1.248	>10
						10	ND	0.372	>10
4	$CH_2CH_2CONH_2$	CH_2(4-F)Ph	$PhCH_2O$	A	$C_{31}H_{33}FN_4O_7 \cdot 1.25H_2O$	14	81,400	0.014	>320
						1A	ND	0.607	>10
						10	ND	0.111	>10
5	$CH_2CH_2CONH_2$	CH_2(3,4-F)Ph	$PhCH_2O$	A	$C_{31}H_{32}F_2N_4O_7 \cdot 0.50H_2O$	14	193,000	0.003	>10
						1A	ND	0.513	>10
						10	ND	0.032	>10
6	$CH_2CH_2CONH_2$	CH_2Cyhexyl	$PhCH_2O$	A	$C_{31}H_{40}N_4O_7 \cdot 1.0H_2O$	14	6,500	0.178	>10
7	$CH_2CH_2CONH_2$	H	$PhCH_2O$	A	$C_{24}H_{28}N_4O_7 \cdot 0.50H_2O$	14	348	>10	>10
8	$CH_2CH_2CONH_2$	CH_2Ph	CH_3	A	$C_{25}H_{30}N_4O_6 \cdot 0.50H_2O$	14	7,660	0.888	>10
9	$CH_2CH_2CONH_2$	CH_2Ph	Cypentyl	A	$C_{29}H_{36}N_4O_6 \cdot 0.50H_2O$	14	37,400	0.034	>10
10	$CH_2CH_2CONH_2$	CH_2Ph		A	$C_{27}H_{32}N_4O_6S_2 \cdot 0.50H_2O$	14	113,650	0.104	>10
						1A	ND	1.18	>10
						10	ND	1.41	>10
11[f]	$CH_2CH_2CONH_2$	CH_2Ph		A	$C_{28}H_{34}N_4O_7 \cdot 0.50TFA$	14	3,750	1.78	>10
12	$CH_2CH_2CONH_2$	CH_2Ph	$C(CH_3)_3$	A	$C_{28}H_{36}N_4O_6 \cdot 0.50H_2O$	14	5,040	0.518	>10
13	$CH_2CH_2CONH_2$	CH_2Ph		A	$C_{28}H_{31}N_5O_7 \cdot 0.50H_2O$	14	329,000	0.016	>10
14	$CH_2CH_2CONH_2$	CH_2Ph		A	$C_{27}H_{28}ClN_5O_7 \cdot 0.60H_2O$	14	133,500	0.024	>10
15		CH_2Ph	$PhCH_2O$	B	$C_{33}H_{36}N_4O_7 \cdot 0.25H_2O$	14	380,000	0.003	>1
16		CH_2Ph	$PhCH_2O$	B	$C_{33}H_{36}N_4O_7 \cdot 0.50H_2O$	14	6,340	0.469	>10
Pirodavir				ref. 49		14	NA	0.044	>10
Pleconaril				ref. 6b		14	NA	0.058	>10

[a] Method of preparation: see Schemes 1 and 2. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Inhibition activity against HRV 3CP; see ref 15 for assay method and error. [d] Antirhinoviral activity; see ref 15 for assay method and error. [e] Cytotoxicity; see ref 15 for assay method and error. [f] 1:1 mixture of diastereomers. ND = not determined. NA = not applicable.

was significantly less potent when examined against two other HRV serotypes in cell culture, suggesting that the improvements observed with type 14 HRV would not be realized against a majority of HRV strains. A variety of other aliphatic *N*-terminal amides could be incorporated into the 2-pyridone-containing inhibitor series to provide active compounds, although most were not as potent as the Cbz-containing molecule originally studied (compare compounds **11** and **12** with **3**, Table 1). However, as was observed in our earlier studies of peptidyl and ketomethylene-containing peptidomimetic 3CP inhibitors, a compound containing an *N*-terminal amide derived from 5-methylisoxazole-3-carboxylic acid (**13**) displayed improved anti-3CP and in vitro antirhinoviral properties relative to the corresponding benzyl carbamate **3** (Table 1).[30] Interestingly, the magnitude of these improvements was not nearly as great as that noted in our previous work. Again, the source of the activity differences between the current series of 2-pyridone 3CP inhibitors and the related peptide and peptidomimetic molecules is not known with certainty. Utilization of a P_4 moiety derived from 5-chloroisoxazole-3-carboxylic acid also afforded a relatively potent 3CP inhibitor and antirhinoviral agent (compound **14**, Table 1) suggesting that the 5-chloro and 5-methyl isoxazole substituents could be accommodated nearly equally in the 3CP active site.

In addition to examining modification of the P_2 and P_4 substituents contained within the lead compound **3**, we also studied variation of the P_1 moiety. Our earlier studies identified an (*S*)-γ-lactam fragment that imparted high levels of 3CP inhibition activity to peptidyl and peptidomimetic molecules in which it was incorporated.[18a] Accordingly, this fragment was introduced into the 3-amino-2-pyridone-containing 3CP inhibitor series of the present work. As expected, the resulting compound displayed improved anti-3CP and antiviral properties relative to the glutamine-containing molecule originally studied (compare **15** with **3**, Table 1). However, as noted above for *N*-terminal isoxazole-containing 2-pyridone 3CP inhibitors, the activity improvements realized by P_1-lactam incorporation were not as great in the 2-pyridone inhibitor series when compared to those observed earlier during our studies of peptide and peptidomimetic compounds. As anticipated from those earlier studies, introduction of the corresponding P_1 (*R*)-γ-lactam into the 2-pyridone-containing inhibitor design resulted in drastic loss of 3CP inhibition activity and antirhinoviral properties when compared with the molecule that contained the (*S*)-lactam isomer (compound **16**, Table 1, compare to **15**).

X-ray Structure Analysis

The iterative analysis of protein−ligand interactions by X-ray crystallography is a critical component of any structure-based inhibitor design program.[31] Accordingly, several crystal structures of 3CP−inhibitor complexes were obtained during the development of the 2-pyridone-containing compounds described above. These structures confirmed the binding geometries that the inhibitors adopted when complexed with 3CP and suggested possibilities for compound modification. As in our earlier work, the X-ray data were utilized somewhat cautiously for inhibitor design since the structures depicted the covalent protein−inhibitor products and not the presumably more relevant transition states for adduct formation (see below). The specific details of one particular protein−inhibitor complex are discussed below.

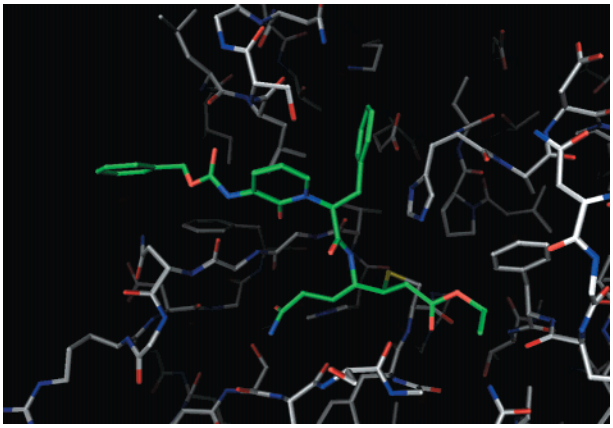


Figure 2. Crystal structure of **3** complexed with HRV-2 3CP (2.3 Å resolution).

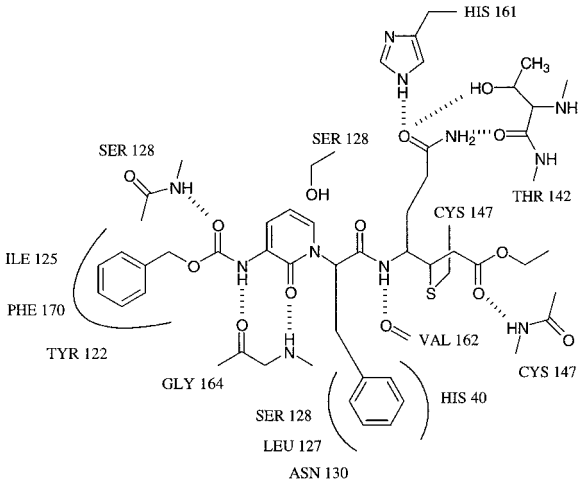


Figure 3. Schematic diagram of **3** bound in the HRV-2 3CP active site. Hydrogen bonds are represented as dashed lines, and the residues that make up the enzyme binding subsites are depicted.

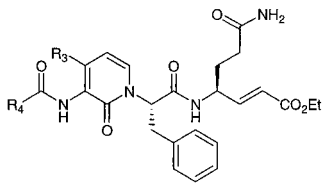
The 2.3 Å X-ray crystal structure of the covalent adduct formed between compound **3** and HRV-2 3CP[32] is shown in Figure 2, and key protein−inhibitor interactions are illustrated in Figure 3. In general, the inhibitor bound to the enzyme in a manner similar to that observed previously for related tripeptidyl molecules[15,29] and filled a series of shallow grooves on the protein surface *N*-terminal to the scissile amide bond of the substrate (S_1−S_4).[14] As expected, a covalent bond was observed between the 3CP active site cysteine residue (Cys-147) and the β-carbon of the Michael acceptor of **3**. As with related peptidyl 3CP inhibitors, a hydrogen bond between the Michael acceptor ester moiety and the 3CP Cys-147 amide NH was present in the above complex, but it is uncertain whether this interaction facilitates addition of the cysteine to **3** or merely arises after the covalent adduct has been formed.

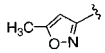
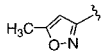
Several other important protein−ligand interactions were apparent in addition to those noted in the vicinity of the active site cysteine, which closely paralleled those observed previously with related tripeptidyl 3CP inhibi-

tors. For example, the glutamine amide of **3** formed hydrogen bonds with the His-161 side chain and both the Thr-142 side chain and the amide carbonyl in the bottom and on the edge of the 3CP S_1 binding pocket, respectively. In addition, the inhibitor phenylalanine residue bound to 3CP in a canyon formed by the side chains of Leu-127, Ser-128, and Asn-130 on one side and His-40 on the other. As expected, the greatest differences between the 3CP binding of the 2-pyridone-containing inhibitor **3** and the related tripeptidyl molecules were noted in the vicinity of the pyridone ring itself. The conformationally mobile serine residue that forms a hydrogen bond with the P_2-P_3 amide NH of most peptidyl 3CP inhibitors (Ser-128) was positioned within van der Waals contact distance adjacent to the pyridone ring (Figure 3). As was noted previously during studies of peptidyl 3CP inhibitors, the *N*-terminal Cbz moiety of **3** was situated in a hydrophobic pocket on the surface of the protein formed by the side chains of Tyr-122, Ile-125, and Phe-170. Also as expected, an antiparallel β-sheet hydrogen-bonding interaction was noted between the pyridone moiety and the Gly-164 (Figure 3), which mimicked that crystallographically observed between the 3CP and the P_3 amino acid residue of related tripeptidyl 3CP inhibitors. The other backbone amide NH present in **3** formed a hydrogen bond with 3CP Val-162, while an additional hydrogen bond between the inhibitor Cbz carbonyl moiety and the 3CP Ser-128 amide NH was also evident (Figure 3). Thus, a total of eight hydrogen bonds involving both the inhibitor amino acid residues and the 2-pyridone moiety were observed in the complex between compound **3** and HRV-2 3CP.

Analysis of the above complex suggested several possibilities for beneficial modification of 3CP inhibitors that contained the 2-pyridone fragment. In particular, the crystal structure indicated that the pyridone$-P_4$ carbamate moiety was significantly twisted out of the plane defined by the pyridone ring. However, molecular mechanics calculations (MM2) conducted with uncomplexed 3-carbamoyl-2-pyridones predicted a coplanar relationship between the carbamate and the heterocyclic ring. Together, these observations suggested that alkyl substitution of the pyridone ring adjacent to the carbamate moiety might minimize the conformational differences between the 3CP-complexed and the uncomplexed structures. This suggestion was supported by ab initio studies conducted at the HF 6-31G* level of theory. The crystal structure analysis also indicated that introduction of such an alkyl moiety should not sterically interfere with the binding of such compounds to the 3CP enzyme. In the event, however, a 3-carbamoyl-4-methyl-2-pyridone-containing compound displayed significantly reduced 3CP inhibition activity when compared to a related nonmethylated molecule (compare **17** with **3**, Table 2). An additional comparison was made between two amide-containing molecules (compounds **13** and **18**, Table 2). Again, the methylated pyridone **18** displayed reduced anti-3CP activity when compared to its nonmethylated analogue **13**, although the difference in activity between the two amide-containing compounds was not as dramatic as that noted above for the benzyl carbamates. The failure of the 4-methyl pyridones to improve 3CP inhibition properties is currently not well understood but may involve uncertainties associated with modeling efforts conducted with irreversible Michael reaction products rather than the transition states of such transformations.

Table 2. 4-Methyl-2-Pyridone-Containing 3CP Inhibitors



Compd No.	R_4	R_3	Prep.[a]	Formula[b]	$k_{obs}/[I]$ ($M^{-1}s^{-1}$)[c]
3	$PhCH_2O$	H	A	$C_{31}H_{34}N_4O_7 \cdot 0.25H_2O$	114,000
17	$PhCH_2O$	CH_3	A	$C_{32}H_{36}N_4O_7 \cdot 0.75H_2O$	25,800
13		H	A	$C_{28}H_{31}N_5O_7 \cdot 0.50H_2O$	329,000
18		CH_3	A	$C_{29}H_{33}N_5O_7 \cdot 1.5TFA$	116,000

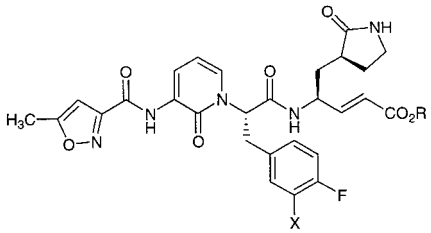
[a] Method of preparation: see Scheme 1. [b] Elemental analyses (C, H, N) of all compounds agreed to within $\pm 0.4\%$ of theoretical values. [c] Inhibition activity against HRV-14 3CP; see ref 15 for assay method and error.

Inhibitor Optimization

On the basis of the experimental results described above, additional inhibitor optimization efforts were conducted utilizing 3-amino-2-pyridones that lacked substituents at the pyridone 4-position. Accordingly, various beneficial functional groups identified during the initial phase of structure−activity relationship studies described above (Table 1) were combined to form new 3CP inhibitors. Thus, a 2-pyridone containing a P_1 (*S*)-γ-lactam moiety, a P_2 benzyl substituent, and an *N*-terminal (P_4) isoxazole fragment displayed very good levels of 3CP inhibition activity as well as extremely potent antirhinoviral properties in cell culture (compound **19**, Table 3). This molecule also exhibited potent antiviral activity against two HRV strains other than type 14. However, the compound was extensively metabolized when incubated with human liver microsomes (Table 3), and additional efforts were therefore made to improve upon the biological stability of the molecule.

Analysis of the incubation mixtures of **19** with human liver microsomes identified several metabolites including compounds derived from oxidation of the 4-fluorophenyl aromatic ring and hydrolysis of the carboxylic acid ethyl ester.[33] In an attempt to reduce the formation of metabolites resulting from the former process, an additional fluorine atom was introduced at the 3-position of the P_2 benzyl substituent present in **19**. The resulting difluorinated compound (**20**) displayed improved anti-3CP and antirhinoviral properties relative to the monofluorinated molecule (Table 3, compare **19** with **20**), and this result was consistent with similar improvements noted previously for other "unoptimized" 2-pyridone 3CP inhibitors (see Table 1, compounds **4**

Table 3. Optimized 2-Pyridone-Containing 3CP Inhibitors



compd no.	R	X	prep[a]	formula[b]	serotype	k_{obs}/[I] (M^{-1} s^{-1})[c]	EC_{50} (μM)[d]	CC_{50} (μM)[e]	HLM (% met)[f]	plasma $T_{1/2}$ (h)[g]
19	CH_2CH_3	H	B	$C_{30}H_{32}FN_5O_7 \cdot 0.50H_2O$	14	1 250 000	0.002	>10	95	ND
					1A	ND	0.015	>10		
					10	ND	0.004	>10		
20	CH_2CH_3	F	B	$C_{30}H_{31}F_2N_5O_7$	14	1 800 000	0.001	>10	80	1.4
					1A	ND	0.006	>10		
					10	ND	0.005	>10		
21	$CH(CH_3)_2$	F	C	$C_{31}H_{33}F_2N_5O_7 \cdot 0.75H_2O$	14	548 000	0.003	>10	55	9.7
					1A	ND	0.132	>10		
					10	ND	0.085	>10		
					2	ND	0.048	>10		
					3	ND	0.033	>10		
					9	ND	0.109	>10		
					16	ND	0.036	>10		
					25	ND	0.100	>10		
					39	ND	0.044	>10		
					13	ND	0.044	>10		
					78	ND	0.012	>10		
					11	ND	0.003	>10		
					19	ND	0.009	>10		
					23	ND	0.010	>10		
					Hanks	ND	0.003	>10		

[a] Method of preparation: see Schemes 2 and 3. [b] Elemental analyses (C, H, N) of all compounds agreed to within ±0.4% of theoretical values. [c] Inhibition activity against HRV 3CP; see ref 15 for assay method and error. [d] Antirhinoviral activity; see ref 15 for assay method and error. [e] Cytotoxicity; see ref 15 for assay method and error. [f] Loss of compound (% metabolized) after 30 min exposure to human liver microsomes; see Experimental Section for additional details. [g] Half-life of compound upon exposure to human plasma; see Experimental Section for additional details.

and **5**). In addition, the difluorinated compound **20** displayed improved stability toward human liver microsomes as compared with **19**, although the metabolism of **20** was still somewhat facile in the in vitro assay (Table 3). Unfortunately, and somewhat surprisingly based on our earlier studies of peptidyl and peptidomimetic 3CP inhibitors,[18a] compound **20** exhibited a short half-life when exposed to human plasma (Table 3), and efforts were accordingly focused on improving this important biological stability parameter.

We anticipated that the instability of compound **20** toward human plasma was primarily due to enzyme-mediated hydrolysis of the ethyl ester contained in the molecule. Accordingly, the ethyl ester was replaced with a sterically more demanding isopropyl ester (compound **21**). This alteration afforded improved performance in the human liver microsomal stability assay and dramatically improved the half-life of the compound in the presence of human plasma (Table 3). These benefits, however, were realized with a simultaneous worsening of anti-3CP and antiviral potency of **21** relative to the ethyl ester-containing molecule **20**. Despite these reductions, compound **21** displayed good absolute levels of antirhinoviral potency when tested against 15 HRV serotypes in cell culture (Table 3). Because this molecule combined good levels of antiviral potency with relatively good biological stability, it was therefore chosen for subsequent oral bioavailability studies.

Oral Bioavailability Studies

The α,β-unsaturated esters contained within tripeptidyl molecules related to the 2-pyridones of the present study were previously shown to be quite unstable toward a variety of rodent plasmas (e.g., compound **1**, $t_{1/2}$ = 10 min in rat plasma).[15] This rodent plasma instability precluded the use of rats and/or mice for oral bioavailability testing of the compounds of the present study.[34] Accordingly, the dog was selected as an appropriate species in which to conduct the desired analysis. In the event, inhibitor **21** displayed good bioavailability and pharmacokinetic properties after oral administration in the dog (Figure 4, Table 4). Encouragingly, plasma levels of the molecule were observed after initial oral dosing, which substantially exceeded the compound's previously determined average in vitro EC_{50} antirhinoviral potency (0.045 μM, 0.028 μg/mL, 15 HRV serotypes), and these levels remained above this average potency for more than 8 h. Because no animal model predictive of rhinovirus-associated human symptomatology has been identified to date, the ultimate significance of the above pharmacokinetic profile cannot be determined with certainty. However, the PK results do demonstrate that 2-pyridone-containing, peptidomimetic 3CP inhibitors can display good oral bioavailability properties in the dog and suggest that additional study of such compounds for use as orally bioavailable HRV 3CP inhibitors is warranted.

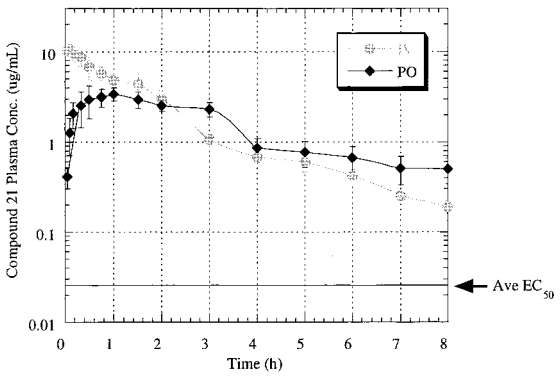
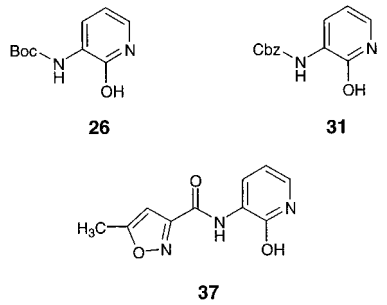


Figure 4. Pharmacokinetic profile of **21** in dogs after IV and oral administration ($n = 3$). Vehicle = 80:20 propylene glycol: H_2O. The average antirhinoviral potency of **21** as determined against 15 HRV serotypes in cell culture is indicated by the arrow. An additional sample was collected 24 h after both IV and PO dosing, but the concentration of **21** was below the limit of detection (5 ng/mL) in each.

Synthesis

The 2-pyridone-containing 3CP inhibitors described in this study were prepared by three related synthetic methods (A, B, and C). The particular method employed to synthesize a given compound is indicated in Tables 1−3, and representative examples of each are given below. These syntheses differ primarily in the nature of the P_1 fragment utilized to begin each sequence and employ several synthetic transformations and intermediates from previously reported preparations of related tripeptidyl 3CP inhibitors.[15,18a] The first method (method A) is illustrated in Scheme 1 with the preparation of compound **13** and involves alkylation of a Boc-protected pyridone entity (P_3 fragment) with an activated P_2-P_1 moiety. Thus, commercially available *N*-α-Cbz-γ-trityl-L-glutamine was reduced to primary alcohol **22** by a two step process, and this entity was subsequently converted to the corresponding *tert*-butyldimethylsilyl ether **23** in good yield. Deprotection of the Cbz moiety present in **23** followed by coupling of the resulting amine (not shown) with commercially available (2*R*)-2-hydroxy-3-phenylpropionic acid afforded intermediate **24** in good yield. The secondary alcohol moiety present in **24** was then converted to the corresponding mesylate (**25**), and the crude material thus obtained was condensed with the sodium salt of (2-hydroxypyridin-3-yl)carbamic acid *tert*-butyl ester (**26**, see Experimental Section) at elevated temperature to afford product **27** as a single diastereomer in moderate yield. Attempts to form the triflate corresponding to **25** utilizing trifluoromethanesulfonic anhydride resulted in the unwanted removal of both the primary silyl ether and the trityl protecting group present in **24**. These side reactions were presumably caused by the formation of trifluoromethanesulfonic acid in the reaction medium and occurred even in the presence of a large excess of various amine bases (e.g., 2,6-lutidine).[35] The synthesis of intermediate **27** predominantly provided the *N*-alkylated pyridone product as evidenced by thin-layer chromatography (TLC) analysis of the reaction mixture and ^{1}H nuclear magnetic resonance (NMR) analysis of the pyridone product.[36,37] The described preparation of intermediate **27** minimized racemization of the stereocenter adjacent to the pyridone ring that was encountered when more direct synthetic methods (e.g., $P_4-P_3-P_2 + P_1$ coupling) were explored.



The silyl protecting group present in **27** was removed, and the resulting alcohol (**28**) was subjected to an oxidation/olefination sequence to provide intermediate **29** in good overall yield. As was encountered during previous syntheses of peptidyl and peptidomimetic 3CP inhibitors,[15,18a] the above olefination process afforded the desired trans isomer with <5% of the corresponding cis isomer as determined by ^{1}H NMR analysis of the crude reaction mixture. Selective removal of the Boc protecting group present in **29** proved to be surprisingly difficult but was eventually accomplished through thermal deprotection. The resulting amine (not shown) was condensed with 5-methylisoxazole-3-carbonyl chloride to provide product **30** in good yield. Subsequent removal of the trityl moiety contained within **30** afforded the desired 3CP inhibitor **13** in good yield after purification by silica gel chromatography.

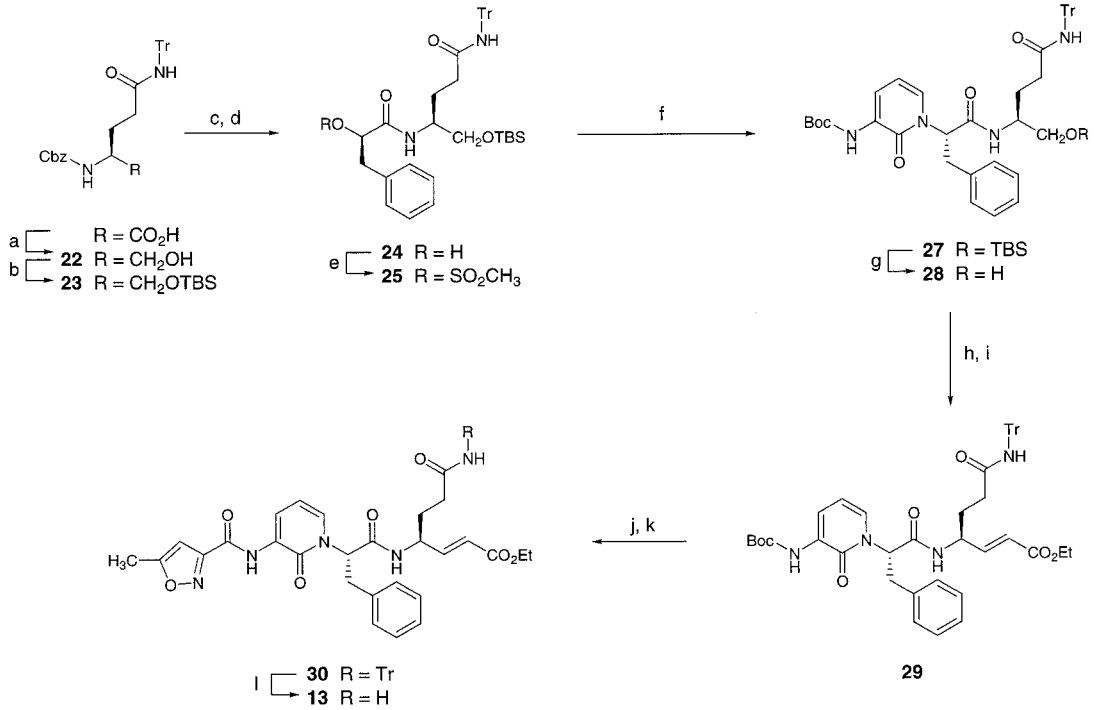
The majority of the other 2-pyridone-containing 3CP inhibitors described in this work could be prepared by variation of synthetic method A described above. Utilization of (2-hydroxypyridin-3-yl)carbamic acid benzyl ester **31**[38] in lieu of **26** allowed for the synthesis of compound **3** (Table 1) without additional manipulation of the 3-amino-pyridone substituent following alkylation of the pyridone moiety. Similarly, incorporation of either Boc- or Cbz-protected 3-amino-2-hydroxy-4-methylpyridine (prepared in analogy to **26** and **31**) into Scheme 1 above (replacing **26**) allowed for the synthesis of inhibitors **17** and **18**. Alternatively, substitution of appropriately functionalized 2-hydroxyacetic acids in place of the (2*R*)-2-hydroxy-3-phenylpropionic acid employed in Scheme 1 above enabled the synthesis of compounds **4**−**7** (Cbz-protected pyridone **31** was also employed). The hydroxy acid required for the preparation of compound **7** was commercially available. The remainder was prepared from the corresponding (commercially available) Boc-protected D-amino acids by a two step process analogous to that described for the synthesis of intermediate **34** below. Finally, replacement of 5-methylisoxazole-3-carbonyl chloride in the Scheme 1 sequence with other carboxylic acids and/or acid chlorides allowed for the preparation of compounds **8**−**14** in addition to inhibitor **13** described above.[39]

The synthesis of 2-pyridone-containing 3CP inhibitors that incorporate P_1-γ-lactam moieties is illustrated in Scheme 2 with the preparation of compound **20** (synthetic method B). Thus, the known[18a] γ-lactam-containing alcohol **32** was converted to the corresponding *tert*-butyldiphenylsilyl ether **33** in good yield. Deprotection

Table 4. Pharmacokinetic Parameters for **21** in Dogs after IV and Oral Administration ($n = 3$)

dose	C_{max} (μg/mL) T_{max} (min)	AUC (0→∞) (μg·min/mL)	CL (mL/min/kg)	V (L/kg)	$T_{1/2}$ (min)	F (%)
15 mg/kg iv	10.6 at 4.0	1028	14.7	4.2	194	
30 mg/kg po	3.5 at 70	980	33	9.6	204	48

Scheme 1[a]



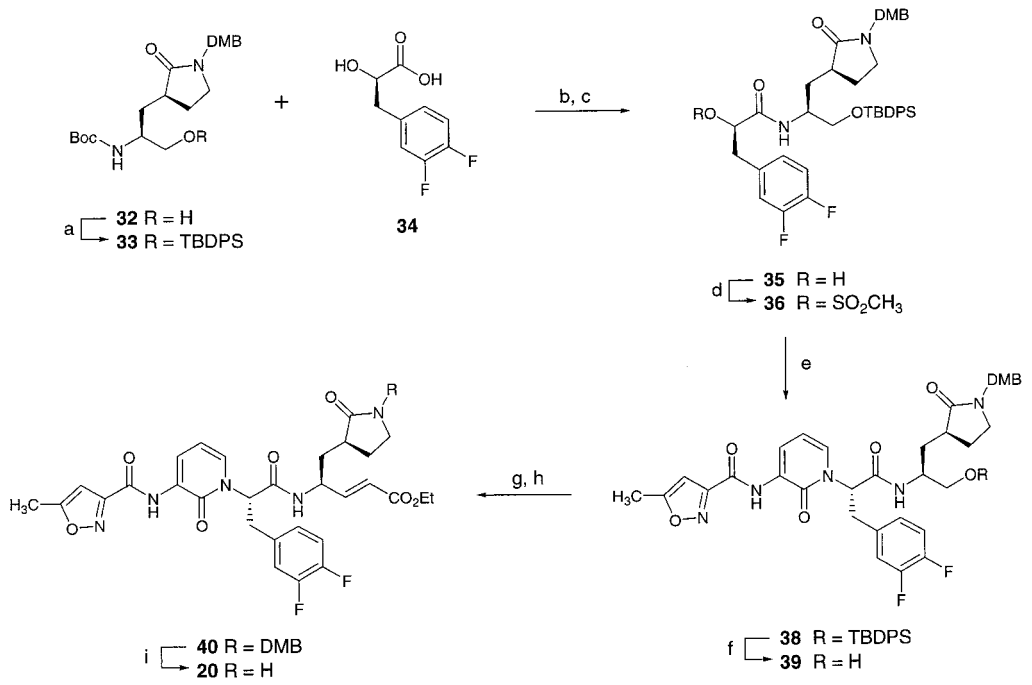
[a] Synthetic method A. Reagents and conditions (Tr = $CHPh_3$, TBS = $Si(CH_3)_2{}^tBu$): (a) 1.0 equiv ethyl chloroformate, 1.0 equiv NMM, THF, −10 °C, 20 min, then 2.25 equiv $NaBH_4$, H_2O, 23 °C, 5 h; (b) 2.3 equiv imidazole, 1.1 equiv TBSCl, DMF, 23 °C, 12 h, 71%; (c) H_2/Pd/C, EtOH, 23 °C, 12 h; (d) 1.0 equiv (2*R*)-2-hydroxy-3-phenylpropionic acid, 2.0 equiv $^iPr_2Net$, 1.0 equiv HATU, DMF, 0→23 °C, 1.5 h, 62%; (e) 1.4 equiv $^iPr_2Net$, 1.2 equiv CH_3SO_2Cl, CH_2Cl_2, −10 °C, 30 min; (f) 2.5 equiv **26**, 2.2 equiv NaH, THF, 0 °C, 20 min, then 1.0 equiv **25**, reflux, 61 h, 77%; (g) $Et_3N{\cdot}3HF$, 1:1 CH_3CN:H_2O, 23 °C, 12 h; (h) 1.1 equiv Dess−Martin periodinane, CH_2Cl_2, 23 °C, 2 h; (i) 1.4 equiv $Ph_3P{=}CHCO_2Et$, THF, reflux 1 h, then 23 °C, 12 h, 77%; (j) 190−200 °C, 1 h; (k) 2.0 equiv 5-methylisoxazole-3-carbonyl chloride, 2.0 equiv NMM, CH_3CN, 0→23 °C, 1 h, 55%; (l) 3.0 equiv $^iPr_3SiH$, 2:3 TFA:CH_2Cl_2, 23 °C, 30 min, 85%.

of the Boc moiety present in **33** under mildly acidic conditions followed by coupling of the resulting amine salt (not shown) with (2*R*)-3-(3′,4′-difluorophenyl)-2-hydroxypropionic acid **34** afforded intermediate **35** in good yield. Carboxylic acid **34** was prepared from Boc-D-3,4-difluorophenylalanine by a two step process analogous to that generally utilized for the conversion of α-amino acids to the corresponding α-hydroxy acids (see Experimental Section).[40] Intermediate **35** was converted to the corresponding mesylate (compound **36**), and this material was condensed in crude form with the sodium salt of pyridone **37** (see Experimental Section) to provide product **38** in good yield. As was observed in the preparation of pyridone **27** in Scheme 1 above, the synthesis of intermediate **38** predominantly provided the *N*-alkylated pyridone product as a single diastereomer.[36,37]

Removal of the silyl protecting group present in **38**, oxidation of the resulting alcohol (**39**), and subsequent olefination of the corresponding aldehyde (not shown) afforded intermediate **40** in moderate yield. The 2,4-dimethoxybenzyl moiety present in **40** was removed by exposure to 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) at elevated temperature to give compound **20**.[41] This deprotection could also be accomplished by exposure of **40** to ceric ammonium nitrate (CAN),[42] although this latter method was somewhat lower yielding than the DDQ deprotection mentioned above. Compounds **15** and **19** were prepared in a manner analogous to that described above for **20** utilizing (2*R*)-2-hydroxy-3-phenylpropionic acid (commercially available) and (2*R*)-3-(4′-fluorophenyl)-2-hydroxypropionic acid (prepared from the corresponding D-amino acid), respectively, in place of intermediate **34**. Similarly, compound **16** was synthesized in a manner analogous to that employed for inhibitor **15** above, beginning with the appropriate *R*-lactam isomer of γ-lactam-containing alcohol **32**.[18a]

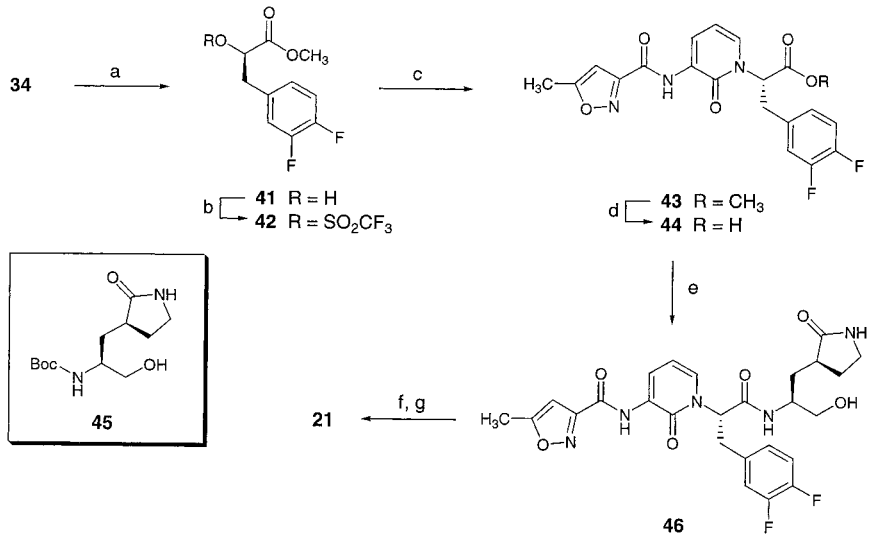
An alternate method for preparing γ-lactam-containing 3CP inhibitors is illustrated in Scheme 3 (method C) and employs an unprotected P_1 moiety (**45**)[43] in place of the dimethoxybenzyl-containing fragment (**32**) utilized in method B above. Method C avoids the need for lactam deprotection in the final step of inhibitor synthesis and therefore enabled more rapid variation of the Michael acceptor moiety incorporated in the compounds under investigation. Surprisingly, several early synthetic intermediates analogous to those depicted in Scheme 2 that lacked the dimethoxybenzyl group proved troublesome to manipulate due to drastically different physical properties. An alternate method for coupling the various inhibitor components together was therefore explored.

Scheme 2[a]



[a] Synthetic method B. Reagents and conditions (DMB = 2,4-dimethoxybenzyl, TBDPS = $SiPh_2tBu$): (a) 5.0 equiv Et_3N, 2.0 equiv TBDPSCl, 0.25 equiv DMAP, CH_2Cl_2, 0→23 °C, 2 h, 50%; (b) HCl, 1,4-dioxane, 23 °C, 1 h; (c) 1.5 equiv **34**, 5.0 equiv NMM, 1.5 equiv HATU, CH_3CN, 0→23 °C, 2.5 h, 54%; (d) 1.4 equiv $^iPr_2NEt$, 1.25 equiv CH_3SO_2Cl, CH_2Cl_2, −10 °C, 30 min; (e) 2.0 equiv **37**, 1.8 equiv NaH, THF, 23 °C, 20 min, then 1.0 equiv **36**, THF, reflux, 16 h, 74%; (f) HF, 30:1 CH_3CN:H_2O, 23 °C, 20 min, 90%; (g) 1.2 equiv Dess−Martin periodinane, CH_2Cl_2, 23 °C, 2 h; (h) 1.3 equiv $Ph_3P{=}CHCO_2Et$, THF, reflux 1.7 h, 63%; (i) 4.2 equiv DDQ, 10:1 $CHCl_3$:H_2O, 60 °C, 4 h, 72%.

Scheme 3[a]



[a] Synthetic method C. Reagents and conditions: (a) HCl, CH_3OH, 23 °C, 20 h; (b) 1.7 equiv 2,6-lutidine, 1.6 equiv Tf_2O, 0 °C, 30 min; (c) 1.1 equiv **37**, 1.0 equiv NaH, THF, 23 °C, 30 min, then 1.0 equiv **42**, THF, 23 °C, 2 h, 81%; (d) 2.5 equiv LiI, pyridine, reflux, 30 min, 96%; (e) (from **45**) 2:3 TFA:CH_2Cl_2, 23 °C, 1 h, then 0.95 equiv **44**, 7.0 equiv NMM, 1.1 equiv HATU, DMF, −10→23 °C, 3 h, 48%; (f) 1.2 equiv Dess−Martin periodinane, CH_2Cl_2, 23 °C,1 h; (g) 1.2 equiv $Ph_3P{=}CHCO_2{}^iPr$, THF, reflux 30 min, 46%.

Thus, esterification of (2*R*)-3-(3′,4′-difluorophenyl)-2-hydroxypropionic acid (**34**) under acidic conditions afforded intermediate **41** in moderate yield (Scheme 3). This material was converted to the corresponding triflate **42** and condensed with the sodium salt of pyridone **37** to give intermediate **43** in good yield after purification on silica gel. The ester present in **43** was converted to the corresponding carboxylic acid (**44**) under near-neutral conditions, and the crude material thus obtained was coupled with γ-lactam **45**[43] to give alcohol **46** in moderate overall yield. Oxidation of alcohol **46** and olefination of the resulting aldehyde (not shown) using (triphenyl-λ^5-phosphanylidene)acetic acid isopropyl ester[44] afforded compound **21** in reasonable yield.

Conclusions

The studies presented above demonstrate that peptidomimetic, 2-pyridone-containing irreversible inhibitors of the HRV 3CP can function as potent, broad-spectrum, orally bioavailable antirhinoviral agents. Such 2-pyridone-containing molecules typically display improved HRV 3CP inhibition activities and antiviral

properties when compared with related tripeptidyl 3CP inhibitors. One particular 2-pyridone-containing compound was shown to be orally bioavailable in the dog ($F = 48\%$). Importantly, the plasma concentrations of this molecule in the dog exceeded its average antirhinoviral activity as determined by cell culture assays (EC_{50}, 15 HRV serotypes) for more than 8 h postadministration. Collectively, these results suggest that additional studies of 2-pyridone-containing HRV 3CP inhibitors are warranted and may lead to the identification of such molecules suitable for clinical development as orally delivered agents.

Experimental Section

General descriptions of experimental procedures, reagent purifications, and instrumentation along with conditions and uncertainties for enzyme and antiviral assays are provided elsewhere.[15] ^{1}H NMR chemical shifts are reported in parts per million (δ) downfield relative to internal tetramethylsilane, and coupling constants are given in Hertz. Abbreviations also apply as follows: HATU [*O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate], HOBt (1-hydroxybenzotriazole hydrate), EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride], CDI (1,1′-carbonyldiimidazole), MTBE (*tert*-butyl methyl ether), and TFA (trifluoroacetic acid). Pirodavir[49] was kindly provided by Janssen Pharmaceuticals. Pleconaril was prepared as described in the literature.[6b]

Representative Example of Preparation Method A. Synthesis of *trans*-(2′*S*,4*S*)-6-Carbamoyl-4-(2′-{3″-[(5‴-methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)hex-2-enoic Acid Ethyl Ester (13). (1*S*)-[1-Hydroxymethyl-3-(tritylcarbamoyl)-propyl]carbamic Acid Benzyl Ester (22). 4-Methylmorpholine (1.89 mL, 17.2 mmol, 1.0 equiv) and ethyl chloroformate (1.65 mL, 17.3 mmol, 1.0 equiv) were added to a mixture of commercially available *N*-α-Cbz-γ-trityl-L-glutamine (9.00 g, 17.2 mmol, 1.0 equiv) in tetrahydrofuran (THF; 23 mL) at −10 °C. After the mixture was stirred for 20 min, the reaction mixture was filtered and the filtrate was added dropwise to a suspension of $NaBH_4$ (1.47 g, 38.9 mmol, 2.25 equiv) in H_2O (10 mL) at 0 °C. The resulting mixture was allowed to warm to 23 °C and stirred for 5 h. It was then cooled again to 0 °C, was quenched by the careful addition of 1 N HCl (30 mL), and then was partitioned between MTBE (500 mL) and brine (2 × 100 mL). The organic phase was dried over Na_2SO_4 and evaporated to provide intermediate **22**, which was used without further purification.

(1*S*)-[1-(*tert*-Butyldimethylsilanyloxymethyl)-3-(tritylcarbamoyl)propyl]carbamic Acid Benzyl Ester (23). Intermediate **22**, prepared above, was dissolved in dimethylformamide (DMF; 10 mL). Imidazole (2.69 g, 39.5 mmol, 2.3 equiv) and *tert*-butyldimethylsilyl chloride (2.86 g, 19.0 mmol, 1.1 equiv) were added. The reaction mixture was stirred overnight, then diluted with MTBE (500 mL), and washed sequentially with 2.5% $KHSO_4$, H_2O, $NaHCO_3$, H_2O, and brine (100 mL each). The organic phase was dried over $MgSO_4$ and evaporated. The residue was purified by flash column chromatography (gradient elution, 25→40% EtOAc in hexanes) to provide **23** (7.6 g, 71%) as a white amorphous solid. IR (cm^{-1}): 3307, 1708, 1660, 1496, 1249. ^{1}H NMR ($CDCl_3$): δ −0.01−0.05 (m, 6H), 0.89 (s, 9H), 1.76−1.93 (m, 2H), 2.29−2.40 (m, 2H), 3.56−3.77 (m, 3H), 5.03−5.16 (m, 3H), 7.00 (s, 1H), 7.18−7.39 (m, 20H). Anal. ($C_{38}H_{46}N_2O_4Si$) C, H, N.

(2′*R*,4*S*)-5-(*tert*-Butyldimethylsilanyloxy)-4-(2′-hydroxy-3′-phenylpropionyl-amino)pentanoic Acid Tritylamide (24). Intermediate **23** (7.6 g, 12 mmol, 1 equiv) and 10% palladium on carbon (0.45 g) were suspended in EtOH (140 mL) at 23 °C and hydrogenated at 50 psi overnight. The reaction mixture was filtered through Whatman no. 3 paper, the paper was washed with EtOH (120 mL), and the combined filtrates were evaporated. The residue was combined with (2*R*)-2-hydroxy-3-phenylpropionic acid (1.42 g, 12.2 mmol, 1.0 equiv), ^{i}Pr$_2$NEt (4.25 mL, 24.4 mmol, 2.0 equiv), and HATU (4.64 g, 12.2 mmol, 1.0 equiv) in DMF (35 mL) at 0 °C. After it was stirred for 1 h, the reaction mixture was allowed to warm to 23 °C and stirred 20 min more. Then, 5% $KHSO_4$ (80 mL) and MTBE (600 mL) were added, and the phases were separated. The organic phase was washed with H_2O (80 mL) and brine (70 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (50% EtOAc in hexanes) to provide **24** as a white foam (4.85 g, 62%). IR (cm^{-1}): 3394, 3295, 1666, 1649, 1519, 1255, 1114, 1085. ^{1}H NMR ($CDCl_3$): δ −0.03−0.02 (m, 6H), 0.85 (s, 9H), 1.70−1.89 (m, 2H), 2.18−2.42 (m, 3H), 2.83 (dd, 1H, $J = 13.8, 8.1$), 3.15 (dd, 1H, $J = 13.8, 4.0$), 3.40 (dd, 1H, $J = 10.0, 4.7$), 3.51 (dd, 1H, $J = 10.0, 3.1$), 3.83−3.94 (m, 1H), 4.17−4.23 (m, 1H), 6.79 (d, 1H, $J = 8.7$), 7.09 (s, 1H), 7.17−7.32 (m, 20H). Anal. ($C_{39}H_{48}N_2O_4Si\cdot 0.30H_2O$) C, H, N.

(1′*S*,2*R*)-Methanesulfonic Acid 1-[1′-(*tert*-Butyldimethylsilanyloxymethyl)-3′-(tritylcarbamoyl)propylcarbamoyl]-2-phenylethyl Ester (25). Intermediate **24** (1.96 g, 3.08 mmol, 1.0 equiv) and ^{i}Pr$_2$NEt (0.752 mL, 4.32 mmol, 1.4 equiv) were dissolved in CH_2Cl_2 (30 mL) and cooled to −10 °C. Methanesulfonyl chloride (0.286 mL, 3.70 mmol, 1.2 equiv) was added slowly (dropwise) with vigorous stirring. After 30 min, the reaction mixture was diluted with CH_2Cl_2 (200 mL), washed with brine (50 mL), dried over Na_2SO_4, and concentrated to provide intermediate **25**, which was used without further purification.

(2-Hydroxypyridin-3-yl)carbamic Acid *tert*-Butyl Ester (26). A suspension of 10% palladium on carbon (0.35 g) and 2-hydroxy-3-nitropyridine (5.00 g, 35.7 mmol, 1.0 equiv) in EtOH (170 mL) was subjected to 1 atm of hydrogen for 16 h. After the reaction vessel was purged with argon, the mixture was filtered through Whatman no. 3 paper and the filtrate was evaporated to give 2-hydroxy-3-aminopyridine, which was used without further purification. This crude material thus obtained was dissolved in THF (100 mL) at 23 °C. Di-*tert*-butyl dicarbonate (7.79 g, 35.7 mmol, 1.0 equiv) was added, and the reaction mixture was heated to reflux for 4 h. Additional di-*tert*-butyl dicarbonate (6.0 g, 27 mmol, 0.8 equiv) was then added, and the reaction mixture was refluxed overnight. The solvent was evaporated, and the residue was purified by flash column chromatography (gradient elution, 50→60% EtOAc in hexanes) to provide **26** as a white solid (6.48 g, 83%). IR (cm^{-1}): 3225, 1725, 1649, 1514. ^{1}H NMR ($CDCl_3$): δ 1.52 (s, 9H), 6.33 (dd, 1H, $J = 7.4, 6.6$), 7.01 (dd, 1H, $J = 6.6, 1.8$), 7.56 (s, 1H), 8.11 (d, 1H, $J = 7.1$), 12.61 (s, 1H). Anal. ($C_{10}H_{14}N_2O_3$) C, H, N.

(1″*S*,2′*S*)-(1-{1′-[1″-(*tert*-Butyldimethylsilanyloxymethyl)-3″-(tritylcarbamoyl)propylcarbamoyl]-2′-phenylethyl}-2-oxo-1,2-dihydropyridin-3-yl)carbamic Acid *tert*-Butyl Ester (27). Hydroxypyridine **26** from above (0.838 g, 3.99 mmol, 1.3 equiv) was stirred in THF (20 mL). Sodium hydride (60% dispersion in mineral oil, 0.148 g, 3.70 mmol, 1.2 equiv) was added. After the mixture was stirred for 20 min, a solution of mesylate **25** (1.0 equiv based on **26**) in THF (15 mL) was added. The resulting mixture was refluxed for 40 h, whereupon TLC showed the reaction to be only 50% complete. In a separate flask, additional sodium hydride (60% dispersion in mineral oil, 0.111 g, 2.78 mmol, 0.9 equiv) was added to a suspension of **26** (0.647 g, 3.08 mmol, 1.0 equiv) in THF (10 mL). After this was stirred for 20 min, this mixture was added to the original reaction vessel and the resulting mixture was refluxed for an additional 21 h and then stirred at 23 °C for 48 h. The crude reaction mixture was diluted with MTBE (600 mL), washed with a mixture of brine and 10% $KHSO_4$ (3:1, 80 mL) and brine (80 mL), then was dried over Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (gradient elution, 30→35% EtOAc in hexanes) to afford **27** as a white foam (1.98 g, 77%). IR (cm^{-1}): 3389, 3307, 1725, 1678, 1649, 1590, 1502. ^{1}H NMR ($CDCl_3$): δ −0.02−0.04 (m, 6H), 0.86 (s, 9H), 1.52 (s, 9H), 1.55−1.88 (m, 2H), 2.08−2.14 (m, 2H), 3.19 (dd, 1H, $J = 13.7, 8.1$), 3.39−3.51 (m, 2H), 3.53 (dd, 1H, $J = 14.2, 7.8$), 3.82−3.93 (m, 1H), 5.60−5.67 (m, 1H), 6.17 (t, 1H, $J = 7.3$), 6.44 (d, 1H, $J = 8.3$),

7.04 (s, 1H), 7.12−7.36 (m, 21H), 7.59 (s, 1H), 7.94 (d, 1H, J = 7.1). Anal. ($C_{49}H_{60}N_4O_6Si$) C, H, N.

(1″*S*,2′*S*)-(1-{1′-[1″-Hydroxymethyl-3″-(tritylcarbamoyl)-propylcarbamoyl]-2′-phenylethyl}-2-oxo-1,2-dihydropyridin-3-yl)carbamic Acid *tert*-Butyl Ester (28). Intermediate **27** (0.96 g, 1.16 mmol, 1.0 equiv) was dissolved in a mixture of CH_3CN (15 mL) and H_2O (1.5 mL) in a plastic tube. Triethylamine trihydrofluoride (11 drops) was added, and the reaction solution was stirred overnight at 23 °C. The mixture was then diluted with EtOAc (400 mL), washed with brine (3 × 40 mL), dried over $MgSO_4$, and concentrated to provide intermediate **28**, which was used without further purification.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-*tert*-Butoxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-phenylpropionylamino]-6-(tritylcarbamoyl)hex-2-enoic Acid Ethyl Ester (29).** Crude alcohol **28** from above was dissolved in CH_2Cl_2 (10 mL) at 23 °C, and Dess−Martin periodinane[45] (Lancaster, 0.545 g, 1.28 mmol, 1.1 equiv) was added. After the mixture was stirred for 2 h, the solvent was evaporated and the residue was repeatedly suspended in toluene and concentrated (40 mL, then 2 × 10 mL) to give a yellow foam. This material was dissolved in THF (17 mL). (Carbethoxymethylene)triphenylphosphorane (0.563 g, 1.62 mmol, 1.4 equiv) was added, and the reaction mixture was heated to reflux for 1 h and then stirred at 23 °C overnight. After the solvent was evaporated, the residue was purified by flash column chromatography (gradient elution, 40→50% EtOAc in hexanes) to provide **29** (0.710 g, 77%). IR (cm^{-1}): 3378, 3284, 1719, 1649, 1596, 1508, 1267. ^1H NMR ($CDCl_3$): δ 1.28 (t, 3H, J = 7.1), 1.47 (s, 9H), 1.54−1.69 (m, 1H), 1.87−2.02 (m, 1H), 2.09−2.22 (m, 2H), 3.12 (dd, 1H, J = 13.7, 7.7), 3.47 (dd, 1H, J = 13.7, 8.1), 4.17 (q, 2H, J = 7.1), 4.43−4.54 (m, 1H), 5.51−5.58 (m, 1H), 5.64 (dd, 1H, J = 15.7, 1.6), 6.12 (t, 1H, J = 7.2), 6.60−6.68 (m, 3H), 7.08−7.31 (m, 21H), 7.51 (s, 1H), 7.90 (d, 1H, J = 7.1). Anal. ($C_{47}H_{50}N_4O_7 \cdot 0.50H_2O$) C, H, N.

***trans*-(2′*S*,4*S*)-4-(2′-{3″-[(5‴-Methylisoxazole-3‴-carbonyl)-amino]-2″-oxo-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)-6-(tritylcarbamoyl)hex-2-enoic Acid Ethyl Ester (30).** Intermediate **29** from above (0.088 g, 0.11 mmol, 1.0 equiv) was heated (neat) to between 190 and 200 °C for 65 min and then cooled to 23 °C. The resulting amine, obtained as a dark residue, was dissolved in CH_3CN (2 mL) and cooled to 0 °C. 5-Methylisoxazole-3-carbonyl chloride (0.033 g, 0.23 mmol, 2.0 equiv) and 4-methylmorpholine (0.025 mL, 0.23 mmol, 2.0 equiv) were added, and the reaction mixture was allowed to warm to 23 °C. After the mixture was stirred for 40 min, a mixture of 10% $KHSO_4$ and brine (1:1, 15 mL) and EtOAc (70 mL) was added. The phases were separated, and the organic phase was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (50% EtOAc in hexanes) to provide **30** (0.049 g, 55%). IR (cm^{-1}): 3331, 1678 (br), 1590, 1525. ^1H NMR ($CDCl_3$): δ 1.28 (t, 3H, J = 7.1), 1.58−1.72 (m, 1H), 1.87−2.03 (m, 1H), 2.10−2.26 (m, 2H), 2.48 (s, 3H), 3.15 (dd, 1H, J = 13.7, 7.8), 3.47 (dd, 1H, J = 13.7, 8.1), 4.17 (q, 2H, J = 7.1), 4.43−4.55 (m, 1H), 5.54−5.61 (m, 1H), 5.65 (dd, 1H, J = 15.8, 1.5), 6.17 (t, 1H, J = 7.3), 6.45 (s, 1H), 6.65 (dd, 1H, J = 15.8, 5.4), 6.72 (s, 1H), 6.84 (d, 1H, J = 8.0), 7.08−7.32 (m, 22 H), 8.35 (dd, 1H, J = 7.3, 1.5), 9.49 (s, 1H). Anal. ($C_{47}H_{46}N_5O_7 \cdot 0.25H_2O$) C, H, N.

***trans*-(2′*S*,4*S*)-6-Carbamoyl-4-(2′-{3″-[(5‴-methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)hex-2-enoic Acid Ethyl Ester (13).** Intermediate **30** (0.047 g, 0.059 mmol, 1.0 equiv) was dissolved in CH_2Cl_2 (3 mL). Triisopropylsilane (0.036 mL, 0.176 mmol, 3.0 equiv) and TFA (2 mL) were added. The bright yellow solution was stirred for 25 min and then diluted with CCl_4 (3 mL), and all of the volatiles were evaporated. The residue was purified by flash column chromatography (3% CH_3OH in CH_2Cl_2) to afford **13** (0.028 g, 85%) as an amorphous foam. IR (cm^{-1}): 3342, 1666 (br), 1590, 1531, 1455. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.1), 1.70−1.84 (m, 1H), 1.85−1.99 (m, 1H), 2.17−2.24 (m, 2H), 2.48 (s, 3H), 3.18 (dd, 1H, J = 13.7, 7.8), 3.50 (dd, 1H, J = 13.7, 8.1), 4.19 (q, 2H, J = 7.1), 4.43−4.54 (m, 1H), 5.68 (dd, 1H, J = 15.7, 1.3), 5.74−5.82 (m, 1H), 6.00 (s, 1H), 6.19 (s, 1H), 6.32 (t, 1H, J = 7.3), 6.46 (s, 1H), 6.69 (dd, 1H, J = 15.7, 5.5), 7.13−7.30 (m, 5H), 7.48 (dd, 1H, J = 7.3, 1.6), 7.62 (d, 1H, J = 7.6), 8.39 (dd, 1H, J = 7.3, 1.6), 9.46 (s, 1H). Anal. ($C_{28}H_{31}N_5O_7 \cdot 0.50H_2O$) C, H, N.

Representative Example of Preparation Method B. Synthesis of *trans*-(2′*S*,3‴″*S*,4*S*)-4-[3′-(3″,4″-Difluorophenyl)-2′-(3‴-{[1‴-(5‴-methylisoxazol-3‴-yl)methanoyl]-amino}-2‴-oxo-2‴*H*-pyridin-1‴-yl)propanoylamino]-5-(2‴″-oxopyrrolidin-3‴″-yl)pent-2-enoic Acid Ethyl Ester (20). (1*S*,3′*S*)-{2-*tert*-Butyldiphenylsilanyloxy)-1-[1′-(2″,4″-dimethoxybenzyl)-2′-oxo-pyrrolidin-3′-ylmethyl]ethyl}-carbamic Acid *tert*-Butyl Ester (33). Triethylamine (7.52 mL, 54.0 mmol, 5.0 equiv), *tert*-butylchlorodiphenylsilane (5.53 mL, 21.6 mmol, 2.0 equiv), and 4-(dimethylamino)pyridine (0.330 g, 2.70 mmol, 0.25 equiv) were added successively to a 0 °C solution of (1*S*,3′*S*)-{1-[1′-(2″,4″-dimethoxybenzyl)-2′-oxopyrrolidin-3′-ylmethyl]-2-hydroxyethyl}carbamic acid *tert*-butyl ester **32**[18a] (4.41 g, 10.8 mmol, 1 equiv) in CH_2Cl_2 (50 mL). The reaction mixture was allowed to warm to 23 °C and stirred for 2 h. It was then diluted with MTBE (400 mL), washed with brine (2 × 100 mL), dried over $MgSO_4$, and evaporated. The residue was purified by flash column chromatography (gradient elution, 30→40% EtOAc in hexanes) to give **33** (3.51 g, 50%) as a white foam; R_f = 0.40 (40% EtOAc in hexanes). IR (cm^{-1}): 3319, 1678, 1508. ^1H NMR ($CDCl_3$): δ 1.05 (s, 9H), 1.42 (s, 9H), 1.44−1.65 (m, 2H), 2.05−2.17 (m, 1H), 2.23−2.35 (m, 1H), 2.44−2.56 (m, 1H), 3.14−3.21 (m, 2H), 3.55−3.68 (m, 2H), 3.69−3.81 (m, 1H), 3.79 (s, 3H), 3.79 (s, 3H), 4.42 (s, 2H), 4.77 (d, 1H, J = 9.3), 6.41−6.46 (m, 2H), 7.09−7.13 (m, 1H), 7.34−7.46 (m, 6H), 7.61−7.67 (m, 4H). Anal. ($C_{37}H_{50}N_2O_6Si$) C, H, N.

(2*R*)-3-(3′,4′-Difluorophenyl)-2-hydroxypropionic Acid (34). Boc-D-3,4-difluorophenylalanine (5.00 g, 16.6 mmol, 1.0 equiv) was stirred for 4 h in a solution of HCl in 1,4-dioxane (4.0 M, 25 mL). Hexanes (25 mL) were added, and the mixture was stirred an additional 20 min. The resulting solid was collected by filtration, air-dried, dissolved in 1 M H_2SO_4 (70 mL), and cooled to −2 °C. A solution of sodium nitrite (40% aq, 8.25 mL) was then added dropwise, keeping the temperature below 10 °C. After addition was complete, the reaction mixture was held at 0 °C for 3 h and then allowed to warm to 23 °C and stir for 16 h more. The reaction mixture was extracted with MTBE (3 × 70 mL), and the organic phases were dried over $MgSO_4$ and concentrated. The residue was subjected to flash column chromatography (3% CH_3OH in CH_2Cl_2 with 0.2% AcOH) to provide **34** (1.36 g) as an oil, which still contained some minor, unidentified impurities.

(1*S*,2‴*R*,3′*S*)-*N*-{2-(*tert*-Butyldiphenylsilanyloxy)-1-[1′-(2″,4″-dimethoxybenzyl)-2′-oxopyrrolidin-3′-ylmethyl]-ethyl}-3‴-(3‴″,4‴″-difluorophenyl)-2‴-hydroxypropionamide (35). Carbamate **33** (2.49 g, 3.85 mmol, 1.0 equiv) was dissolved in 1,4-dioxane (16 mL) at 23 °C. A solution of HCl in the same solvent (4.0 M, 16 mL) was added. After the solution was stirred for 75 min at 23 °C, the volatiles were evaporated under reduced pressure. The resulting residue was dissolved in CH_3CN (20 mL) and cooled to 0 °C. Hydroxyacid **34** (1.17 g, 5.79 mmol, 1.5 equiv), 4-methylmorpholine (2.12 mL, 19.3 mmol, 5.0 equiv), and HATU (2.20 g, 5.79 mmol, 1.5 equiv) were added sequentially, and the reaction mixture was allowed to warm to 23 °C and then stirred for 2.5 h. It was then partitioned between EtOAc (500 mL) and brine (2 × 125 mL), and the organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (gradient elution, 2→2.5% CH_3OH in CH_2Cl_2) to provide **35** (1.07 g, 54%) as a white foam; R_f = 0.21 (50% EtOAc in hexanes). IR (cm^{-1}): 3389, 3319, 1660, 1514. ^1H NMR ($CDCl_3$): δ 1.05 (s, 9H), 1.54−1.67 (m, 2H), 2.01−2.12 (m, 1H), 2.20−2.40 (m, 2H), 2.78 (dd, 1H, J = 14.1, 7.9), 3.08 (dd, 1H, J = 14.1, 3.7), 3.14−3.21 (m, 2H), 3.50 (dd, 1H, J = 10.1, 5.7), 3.62 (dd, 1H, J = 10.1, 3.7), 3.77 (s, 3H), 3.78 (s, 3H), 3.89 (d, 1H, J = 4.6), 3.96−4.10 (m, 1H), 4.21−4.28 (m, 1H), 4.36 (s, 2H), 6.37−6.44 (m, 2H), 6.89−6.95 (m, 2H),

7.02−7.09 (m, 2H), 7.31−7.45 (m, 7H), 7.60−7.65 (m, 4H). Anal. ($C_{41}H_{48}F_2N_2O_6Si$) C, H, N.

5-Methylisoxazole-3-carboxylic Acid (2′-Hydroxypyridin-3′-yl)amide (37). Palladium, 10% on activated carbon (0.45 g), and 2-hydroxy-3-nitropyridine (7.00 g, 50.0 mmol, 1.0 equiv) in EtOH (210 mL) were subjected to 1 atm of hydrogen for 16 h. After the reaction vessel was purged with argon, the mixture was filtered through Whatman no. 3 paper and the filtrate was evaporated to give 2-hydroxy-3-aminopyridine, which was used without further purification. This crude material was suspended in CH_3CN (170 mL) and cooled to 0 °C. 5-Methylisoxazole-3-carbonyl chloride (8.00 g, 55.0 mmol, 1.0 equiv) was added in one portion. After 25 min at 0 °C, the reaction mixture was allowed to warm to 23 °C and stirred for an additional 75 min. The thick mixture was then poured into dilute HCl (0.02 M, 150 mL) and mixed thoroughly. The undissolved solid was collected by filtration, washed with H_2O (2 × 20 mL), and then dried under vacuum overnight to provide **37** (7.1 g, 65%) as a white solid; mp 270−272 °C, dec; R_f = 0.38 (10% CH_3OH in $CHCl_3$). IR (cm^{-1}): 3460, 3331, 1637, 1543. ^1H NMR (DMSO-d_6): δ 2.48 (s, 3H), 6.29 (dd, 1H, J = 7.2, 6.6), 6.69 (s, 1H), 7.19 (dd, 1H, J = 6.6, 1.8), 8.26 (dd, 1H, J = 7.2, 1.8), 9.43 (s, 1H), 12.20 (s, 1H). Anal. ($C_{10}H_9N_3O_3$) C, H, N.

(1‴*S*,2″*R*,3‴″*S*)-5-Methylisoxazole-3-carboxylic Acid {1′-[1″-{2‴-(*tert*-Butyldiphenylsilanyloxy)-1‴-[1‴″-(2‴″,4‴″-dimethoxybenzyl)-2‴″-oxopyrrolidin-3‴″-ylmethyl]ethylcarbamoyl}-2″-(3‴″″,4‴″″-difluorophenyl)ethyl]-2′-oxo-1′,2′-dihydropyridin-3′-yl}amide (38). *N*,*N*-Diisopropylethylamine (0.357 mL, 2.05 mmol, 1.4 equiv) and **35** (1.07 g, 1.46 mmol, 1.0 equiv) were combined in CH_2Cl_2 (20 mL) and cooled to −10 °C. Methanesulfonyl chloride (0.142 mL, 1.83 mmol, 1.25 equiv) was added slowly (dropwise) with vigorous stirring. After 30 min, the reaction mixture was diluted with MTBE (250 mL), washed sequentially with a mixture of brine and 10% $KHSO_4$ (1:1, 60 mL) and brine (60 mL), dried over Na_2SO_4, and concentrated to give the crude mesylate **36**, which was used without further purification.

Sodium hydride (60% dispersion in mineral oil, 0.105 g, 2.62 mmol, 1.8 equiv) was added to a solution of **37** (0.642 g, 2.93 mmol, 2.0 equiv) in THF (7 mL) at 23 °C. After the mixture was stirred for 20 min, a solution of the crude mesylate **36** (prepared above) in THF (7 mL) was added. The resulting mixture was refluxed for 16 h, cooled to 23 °C, diluted with MTBE (20 mL), and washed sequentially with a mixture of brine and 10% $KHSO_4$ (3:1, 30 mL) and brine (30 mL). The organic layer was dried over Na_2SO_4 and was concentrated. The resulting residue was purified by flash column chromatography (gradient elution, 2→2.5% CH_3OH in CH_2Cl_2) to provide **38** (1.00 g, 74%) as a white foam; R_f = 0.62 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3331, 3295, 1666, 1649, 1596, 1525. ^1H NMR ($CDCl_3$): δ 1.03 (s, 9H), 1.43−1.54 (m, 1H), 1.55−1.67 (m, 1H), 1.90−2.02 (m, 1H), 2.05−2.26 (m, 2H), 2.50 (s, 3H), 3.02−3.17 (m, 3H), 3.43 (dd, 1H, J = 14.1, 7.3), 3.51 (dd, 1H, J = 10.1, 5.8), 3.70 (dd, 1H, J = 10.1, 3.7), 3.76 (s, 3H), 3.78 (s, 3H), 3.95−4.07 (m, 1H), 4.25 (d, 1H, J = 14.6), 4.36 (d, 1H, J = 14.6), 5.50−5.58 (m, 1H), 6.19−6.26 (m, 1H), 6.39−6.47 (m, 3H), 6.78−6.84 (m, 1H), 6.86−7.05 (m, 3H), 7.12−7.17 (m, 1H), 7.32−7.45 (m, 6H), 7.54−7.64 (m, 5H), 8.36−8.40 (m, 1H), 9.56 (s, 1H). Anal. ($C_{51}H_{55}F_2N_5O_8Si$) C, H, N.

(1″*S*,1‴*S*,3‴″*S*)-5-Methylisoxazole-3-carboxylic Acid [1′-(2″-(3‴,4‴-Difluorophenyl)-1‴-{1‴″-[1‴″-(2‴″,4‴″-dimethoxybenzyl)-2‴″-oxopyrrolidin-3‴″-ylmethyl]-2‴″-hydroxyethylcarbamoyl}ethyl)-2′-oxo-1′,2′-dihydropyridin-3′-yl]amide (39). Hydrofluoric acid (48%, 2 mL) was added dropwise to a stirred solution of **38** (0.929 g, 0.997 mmol, 1.0 equiv) in a mixture of CH_3CN (14 mL) and H_2O (0.45 mL) in a plastic tube at 23 °C. After 1.5 h, more hydrofluoric acid (2 mL) was added. After 3 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ (125 mL), extracted with CH_2Cl_2 (3 × 200 mL), dried over Na_2SO_4, and evaporated. The residue was purified by flash column chromatography (gradient elution, 2→3% CH_3OH in CH_2Cl_2) to provide **39** (0.621 g, 90%) as a white foam; R_f = 0.25 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3366 br, 3331, 1672 br, 1596, 1531 br. ^1H NMR ($CDCl_3$): δ 1.48−1.62 (m, 2H), 1.91−2.19 (m, 2H), 2.30−2.40 (m, 1H), 2.49 (s, 3H), 3.10−3.21 (m, 3H), 3.45−3.56 (m, 3H), 3.75 (s, 3H), 3.78 (s, 3H), 3.89−4.05 (m, 2H), 4.17 (d, 1H, J = 14.4), 4.38 (m, 1H, J = 14.4), 5.59−5.68 (m, 1H), 6.20−6.27 (m, 1H), 6.38−6.45 (m, 3H), 6.81−6.87 (m, 1H), 6.91−7.02 (m, 3H), 7.24−7.29 (m, 1H), 8.19−8.25 (m, 1H), 8.36−8.41 (m, 1H), 9.52 (s, 1H). Anal. ($C_{35}H_{37}F_2N_5O_8$) C, H, N.

***trans*-(2′*S*,3‴″*S*,4*S*)-4-(3′-(3″,4″-Difluorophenyl)-2′-{3‴-[(5‴″-methylisoxazole-3‴″-carbonyl)-amino]-2‴″-oxo-2‴*H*-pyridin-1‴″-yl}propionylamino)-5-[1‴″-(2‴″″,4‴″″-dimethoxybenzyl)-2‴″″-oxopyrrolidin-3‴″″-yl]pent-2-enoic Acid Ethyl Ester (40).** Dess−Martin periodinane[45] (Lancaster, 0.390 g, 0.913 mmol, 1.2 equiv) and **39** (0.528 g, 0.761 mmol, 1.0 equiv) were combined in CH_2Cl_2 (8 mL) and stirred for 2 h at 23 °C. The volatiles were then removed under reduced pressure. The residue was suspended in toluene and concentrated to dryness (2 × 10 mL), and the resulting residue was dissolved in THF (16 mL). (Carbethoxymethylene)triphenylphosphorane (0.345 g, 0.990 mmol, 1.3 equiv) was added, and the reaction mixture was refluxed for 1.75 h. It was allowed to cool to 23 °C and then was concentrated. The residue was purified by flash column chromatography (2% CH_3OH in CH_2Cl_2) to provide **40** (0.363 g, 63%) as a foam; R_f = 0.36 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3331, 3284, 1713, 1666, 1649, 1596. ^1H NMR ($CDCl_3$): δ 1.27 (t, 3H, J = 7.1), 1.46−1.65 (m, 2H), 1.85−1.97 (m, 1H), 2.10−2.21 (m, 1H), 2.35−2.47 (m, 1H), 2.50 (s, 3H), 3.10−3.22 (m, 3H), 3.50 (dd, 1H, J = 13.9, 7.5), 3.75 (s, 3H), 3.77 (s, 3H), 4.12−4.21 (m, 3H), 4.38 (d, 1H, J = 14.5), 4.44−4.55 (m, 1H), 5.56−5.63 (m, 1H), 5.73 (dd, 1H, J = 15.6, 1.5), 6.21−6.27 (m, 1H), 6.38−6.47 (m, 3H), 6.73 (dd, 1H, J = 15.6, 5.6), 6.84−6.90 (m, 1H), 6.92−7.07 (m, 3H), 7.22−7.26 (m, 1H), 8.37−8.41 (m, 1H), 8.69 (d, 1H, J = 6.4), 9.55 (s, 1H). Anal. ($C_{39}H_{41}F_2N_5O_9 \cdot 0.75H_2O$) C, H, N.

***trans*-(2′*S*,3‴″*S*,4*S*)-4-[3′-(3″,4″-Difluorophenyl)-2′-(3‴-{[1‴″-(5‴″-methylisoxazol-3‴″-yl)methanoyl]amino}-2‴″-oxo-2‴*H*-pyridin-1‴″-yl)propanoylamino]-5-(2‴″″-oxopyrrolidin-3‴″″-yl)pent-2-enoic Acid Ethyl Ester (20).** DDQ (0.130 g, 0.573 mmol, 1.4 equiv) was added to a solution of **40** (0.312 g, 0.410 mmol, 1.0 equiv) in a mixture of $CHCl_3$ and H_2O (10:1, 11 mL) at 23 °C. The reaction vessel was placed in an oil bath and was heated to 60 °C. After the mixture was stirred for 1.5 h, more DDQ (0.130 g, 0.573 mmol, 1.4 equiv) was added. After an additional 1.5 h, more DDQ (0.130 g, 0.573 mmol, 1.4 equiv) was added. After 4 h total time, the reaction mixture was allowed to cool to 23 °C, was diluted with EtOAc (300 mL), and was washed sequentially with a mixture of 10% $KHSO_4$ and brine (1:1, 100 mL) and a mixture of saturated $NaHCO_3$ and brine (1:1, 100 mL). The organic layer was dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (2% CH_3OH in CH_2Cl_2) to provide **20** (0.181 g, 72%) as a white foam; R_f = 0.43 (10% CH_3OH in $CHCl_3$). IR (cm^{-1}): 3331, 1690, 1649, 1596, 1531, 1455, 1278. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.1), 1.44−1.54 (m, 1H), 1.63−1.78 (m, 1H), 2.08−2.29 (m, 3H), 2.49 (d, 3H, J = 0.9), 3.05 (dd, 1H, J = 13.6, 7.5), 3.20−3.42 (m, 3H), 4.19 (dq, 2H, J = 7.1, 1.7), 4.34−4.45 (m, 1H), 5.64 (dd, 1H, J = 15.7, 1.4), 6.00 (t, 1H, J = 7.8), 6.32 (t, 1H, J = 7.3), 6.45 (s, 1H), 6.71 (dd, 1H, J = 15.7, 5.6), 6.86−6.91 (m, 1H), 6.98−7.08 (m, 2H), 7.15 (s, 1H), 7.68 (dd, 1H, J = 7.3, 1.7), 8.39 (dd, 1H, J = 7.3, 1.7), 8.65 (d, 1H, J = 6.8), 9.46 (s, 1H). Anal. ($C_{30}H_{31}F_2N_5O_7$) C, H, N.

Preparation Method C. Synthesis of *trans*-(2′*S*,3‴″*S*,4*S*)-4-[3′-(3″,4″-Difluorophenyl)-2′-(3‴-{[1‴″-(5‴″-methylisoxazol-3‴″-yl)methanoyl]amino}-2‴″-oxo-2‴*H*-pyridin-1‴″-yl)propanoylamino]-5-(2‴″″-oxopyrrolidin-3‴″″-yl)-pent-2-enoic Acid Isopropyl Ester (21). (2*R*)-3-(3′,4′-Difluorophenyl)-2-hydroxypropionic Acid Methyl Ester (41). A solution of HCl in 1,4-dioxane (0.100 mL) and **34** (0.170 g, 0.841 mmol, 1 equiv) were combined in CH_3OH (5 mL) at 23 °C and maintained for 20 h at that temperature. The reaction mixture was then concentrated under reduced pressure, and the resulting residue was purified by flash column chroma-

tography (25% EtOAc in hexanes) to provide **41** (0.104 g, 57%) as a white solid; mp 49−50 °C; R_f = 0.40 (40% EtOAc in hexanes). IR (cm^{-1}): 3465, 1737. ^1H NMR ($CDCl_3$): δ 2.83 (d, 1H, J = 5.5), 2.91 (dd, 1H, J = 14.0, 6.6), 3.09 (dd, 1H, J = 14.0, 4.2), 3.79 (s, 3H), 4.40−4.47 (m, 1H), 6.90−6.96 (m, 1H), 7.02−7.13 (m, 2H). Anal. ($C_{10}H_{10}F_2O_3$) C, H, N.

(2*R*)-3-(3′,4′-Difluorophenyl)-2-trifluoromethanesulfonyloxypropionic Acid Methyl Ester (42). A solution of **41** (0.091 g, 0.421 mmol, 1.0 equiv) and 2,6-lutidine (0.083 mL, 0.713 mmol, 1.7 equiv) was cooled to 0 °C. Trifluoromethanesulfonic anhydride (0.113 mL, 0.672 mmol, 1.6 equiv) was added dropwise. The reaction mixture was stirred 30 min at 0 °C, then was diluted with MTBE (90 mL), and washed sequentially with 1 M HCl (20 mL) and brine (2 × 20 mL). The organic layer was dried over $MgSO_4$ and concentrated to provide crude **42**, which was used without further purification.

(2*S*)-3-(3′,4′-Difluorophenyl)-2-{3″-[(5‴-methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}propionic Acid Methyl Ester (43). A solution of **37** (0.102 g, 0.465 mmol, 1.1 equiv) in THF was treated with sodium hydride (60% suspension in mineral oil, 0.017 g, 0.425 mmol, 1.0 equiv) at 23 °C. The reaction mixture was stirred for 30 min at that temperature while gas evolution ceased, and then, a THF solution (4 mL) of crude **42** prepared above was added rapidly over 2 min. The resulting reaction mixture was stirred for 2 h at 23 °C, then was partitioned between MTBE (100 mL) and brine (2 × 20 mL). The organic layer was dried over $MgSO_4$ and was concentrated. The residue was purified by flash column chromatography (40% EtOAc in hexanes) to provide **43** (0.142 g, 81%) as a white foam; R_f = 0.29 (40% EtOAc in hexanes). IR (cm^{-1}): 3342, 1743, 1690, 1649, 1602. ^1H NMR ($CDCl_3$): δ 2.50 (s, 3H), 3.33 (dd, 1H, J = 14.4, 9.9), 3.52 (dd, 1H, J = 14.4, 5.5), 3.77 (s, 3H), 5.32 (dd, 1H, J = 9.9, 5.5), 6.17−6.23 (m, 1H), 6.47−6.49 (m, 1H), 6.66−6.82 (m, 2H), 6.89−7.07 (m, 2H), 8.40−8.44 (m, 1H), 9.56 (s, 1H). Anal. ($C_{20}H_{17}F_2N_3O_5$) C, H, N.

(2*S*)-3-(3′,4′-Difluorophenyl)-2-{3″-[(5‴-methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}propionic Acid (44). Lithium iodide (1.15 g, 8.59 mmol, 2.5 equiv) and **43** (1.43 g, 3.43 mmol, 1.0 equiv) were combined in pyridine (6 mL) and refluxed for 30 min. After the mixture was cooled to 23 °C, the reaction mixture was partitioned between 1 M HCl (150 mL) and CH_2Cl_2 (3 × 150 mL). The combined organic phases were washed with a mixture of brine and 1 M HCl (10:1, 3 × 25 mL), then dried over $MgSO_4$, and concentrated to provide the crude carboxylic acid **44** (1.33 g, 96%), which was used without further purification.

(1‴*S*,1⁗*S*,3⁗⁗*S*)-5-Methylisoxazole-3-carboxylic Acid (1′-{2″-(3‴,4‴-Difluorophenyl)-1″-[2⁗-hydroxy-1⁗-(2⁗⁗-oxopyrrolidin-3⁗⁗-ylmethyl)ethylcarbamoyl]ethyl}-2′-oxo-1′,2′-dihydropyridin-3′-yl)amide (46). TFA (4 mL) and **45**[43] (0.591 g, 2.29 mmol, 1.0 equiv) were combined in CH_2Cl_2 (6 mL) and stirred for 50 min. The volatiles were then removed under reduced pressure, and the residue was evaporated repeatedly from CCl_4 (3 × 25 mL). The resulting residue and crude **44** (0.877 g, 2.17 mmol, 0.95 equiv) were combined in DMF (10 mL) and cooled to −10 °C. 4-Methylmorpholine (1.76 mL, 16.0 mmol, 7.0 equiv) and HATU (0.957 g, 2.52 mmol, 1.1 equiv) were added. After the mixture was stirred for 2 h at −10 °C, the vessel was allowed to warm to 23 °C and stirred an additional 1 h. 10% $KHSO_4$ (30 mL), brine (50 mL), and EtOAc (650 mL) were added. The phases were separated, and the organic phase was washed sequentially with brine (30 mL), a mixture of saturated $NaHCO_3$ and brine (1:1, 50 mL), and brine (25 mL), then dried over $MgSO_4$, and concentrated. The residue was purified by flash column chromatography (gradient elution, 3→5% CH_3OH in $CHCl_3$) to provide **46** (0.566 g, 48%) as a white foam; R_f = 0.38 (10% CH_3OH in $CHCl_3$). IR (cm^{-1}): 3331, 1678 br, 1590, 1531. ^1H NMR ($CDCl_3$): δ 1.42−1.53 (m, 1H), 1.64−1.80 (m, 1H), 1.98−2.10 (m, 1H), 2.19−2.32 (m, 2H), 2.48 (s, 3H), 3.12 (dd, 1H, J = 14.0, 8.2), 3.19−3.33 (m, 2H), 3.43 (dd, 1H, J = 14.0, 7.4), 3.52 (s, 2H), 3.80−3.88 (m, 1H), 3.92−4.04 (m, 1H), 5.78−5.85 (m, 1H), 6.25−6.32 (m, 1H), 6.44 (s, 1H), 6.72 (s, 1H), 6.80−6.88 (m, 1H), 6.92−7.02 (m, 2H), 7.44−7.49 (m, 1H), 8.17 (d, 1H, J = 7.9), 8.33−8.40 (m, 1H), 9.44 (s, 1H). Anal. ($C_{26}H_{27}F_2N_5O_6 \cdot 1.0H_2O$) C, H, N.

***trans*-(2′*S*,3⁗⁗*S*,4*S*)-4-[3′-(3″,4″-Difluorophenyl)-2′-(3‴-{[1⁗⁗-(5⁗⁗-methylisoxazol-3⁗⁗-yl)methanoyl]amino}-2‴-oxo-2‴*H*-pyridin-1‴-yl)propanoylamino]-5-(2⁗⁗-oxopyrrolidin-3⁗⁗-yl)-pent-2-enoic Acid Isopropyl Ester (21).** Dess−Martin periodinane[45] (Lancaster, 0.267 g, 0.625 mmol, 1.15 equiv) was added to a 0 °C solution of **46** (0.295 g, 0.543 mmol, 1 equiv) in CH_2Cl_2 (10 mL). The reaction vessel was then warmed to 23 °C and stirred for 45 min. The volatiles were evaporated, and the residue was repeatedly concentrated from toluene (3 × 5 mL). The residue was then dissolved in THF (10 mL). (Triphenyl-λ^5-phosphanylidene)acetic acid isopropyl ester[44] (0.226 g, 0.624 mmol, 1.15 equiv) was added, and the reaction mixture was refluxed for 30 min, then cooled to 23 °C, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (2% CH_3OH in $CHCl_3$) to afford **21** contaminated with several impurities. This material was dissolved in $CHCl_3$ (100 mL), washed sequentially with a mixture of saturated $NaHCO_3$ and brine (1:1, 40 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$ and concentrated to give pure **21** (0.155 g, 46%) as a pale yellow foam; R_f = 0.29 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3507, 3331, 3284, 1690, 1649, 1596, 1525. ^1H NMR ($CDCl_3$): δ 1.27 (d, 3H, J = 6.2), 1.28 (d, 3H, J = 6.2), 1.45−1.56 (m, 1H), 1.62−1.78 (m, 1H), 2.08−2.29 (m, 3H), 2.49 (s, 3H), 3.06 (dd, 1H, J = 13.8, 7.8), 3.21−3.44 (m, 3H), 4.36−4.47 (m, 1H), 4.99−5.12 (m, 1H), 5.66 (dd, 1H, J = 15.6, 1.5), 5.96−6.04 (m, 1H), 6.32 (t, 1H, J = 7.3), 6.45 (s, 1H), 6.71 (dd, 1H, J = 15.6, 5.6), 6.84−6.91 (m, 1H), 6.98−7.17 (m, 3H), 7.66 (dd, 1H, J = 7.3, 1.6), 8.39 (dd, 1H, J = 7.3, 1.6), 8.64 (d, 1H, J = 6.6), 9.45 (s, 1H). Anal. ($C_{31}H_{33}F_2N_5O_7 \cdot 0.75H_2O$) C, H, N.

The following compounds were prepared using modifications of either general synthetic method A, B, or C as indicated in Tables 1−3.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-phenylpropionylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (3).** R_f = 0.28 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3298, 1713, 1655, 1590, 1508, 1196. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.1), 1.65−1.96 (m, 2H), 2.02−2.19 (m, 2H), 3.15 (dd, 1H, J = 13.8, 7.6), 3.49 (dd, 1H, J = 13.8, 8.3), 4.18 (q, 2H, J = 7.1), 4.42−4.53 (m, 1H), 5.17 (s, 2H), 5.62−5.81 (m, 4H), 6.26−6.52 (m, 1H), 6.66 (dd, 1H, J = 15.6, 5.4), 7.12−7.40 (m, 12H), 7.81 (s, 1H), 7.97−8.04 (m, 1H). Anal. ($C_{31}H_{34}N_4O_7 \cdot 0.25H_2O$) C, H, N.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-(4‴-fluorophenyl)propionylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (4).** mp 99−101 °C; R_f = 0.66 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3308, 1714, 1650, 1511, 1199. ^1H NMR ($CDCl_3$): δ 1.33 (t, 3H, J = 6.9), 1.72−1.82 (m, 1H), 1.86−1.96 (m, 1H), 2.10−2.18 (m, 2H), 3.10−3.17 (m, 2H), 3.10−3.17 (m, 2H), 3.46−3.53 (m, 1H), 3.63−3.74 (m, 2H), 4.22 (q, 2H, J = 6.9), 4.48−4.57 (m, 1H), 5.21 (s, 2H), 5.58−5.65 (m, 2H), 5.91−5.97 (m, 1H), 6.19−6.22 (m, 1H), 6.35 (t, 1H, J = 7.2), 6.69 (dd, 1H, J = 15.6, 5.1), 6.98 (t, 2H, J = 8.7), 7.13−7.18 (m, 2H), 7.36−7.40 (m, 5H), 7.85 (s, br. 1H), 8.06−8.09 (m, 1H). Anal. ($C_{33}H_{33}N_4O_7 \cdot 1.25\ H_2O$) C, H, N.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-(3‴,4‴-difluorophenyl)propionylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (5).** mp 175−178 °C; R_f = 0.43 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3298, 1661, 1516, 1266. ^1H NMR ($CDCl_3$): δ 1.32 (t, 3H, J = 7.2), 1.74−1.95 (m, 2H), 2.12−2.20 (m, 2H), 3.05−3.12 (m, 1H), 3.42−3.50 (m, 1H), 4.21 (q, 2H, J = 7.2), 4.45−4.56 (m, 1H), 5.21 (s, 2H), 5.58−5.71 (m, 3H), 6.07−6.10 (m, 1H), 6.32−6.34 (m, 1H), 6.71 (dd, 1H, J = 15.6, 5.1), 6.88−6.91 (m, 1H), 6.99−7.11 (m, 2H), 7.30−7.33 (m, 1H), 7.36−7.39 (m, 5H), 7.53 (s, br. 1H), 7.86 (s, br. 1H), 8.04 (s, br. 1H). Anal. ($C_{31}H_{32}N_4O_7 \cdot 0.50H_2O$) C, H, N.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-cyclohexylpropionylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (6).** mp 64−66 °C;

R_f = 0.47 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3302, 2925, 1721, 1651, 1197. ^1H NMR ($CDCl_3$): δ 0.93−1.05 (m, 2H), 1.18−1.22 (m, 4H), 1.32 (t, 3H, J = 7.2), 1.66−1.75 (m, 4H), 1.82−1.91 (m, 2H), 1.94−2.06 (m, 2H), 2.10−2.17 (m, 2H), 2.90−2.92 (m, 2H), 4.23 (q, 2H, J = 7.2), 4.52−4.60 (m, 1H), 5.23 (s, 2H), 5.65 (t, 1H, J = 8.1), 5.82−5.86 (m, 1H), 5.62 (dd, 1H, J = 15.9, 1.8), 6.01−6.05 (m, 1H), 6.37 (t, 1H, J = 7.2), 6.85 (dd, 1H, J = 15.9, 5.7), 7.25−7.29 (m, 1H), 7.36−7.43 (m, 5H), 7.88 (s, br. 1H), 8.09 (d, 1H, J = 6.9). Anal. ($C_{31}H_{40}N_4O_7$·1.0H_2O) C, H, N.

***trans*-(4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-acetylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (7).** mp 169−174 °C; R_f = 0.24 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3273, 1719, 1649. ^1H NMR (DMSO-d_6): δ 1.19 (t, 3H, J = 7.1), 1.64−1.85 (m, 2H), 2.10 (t, 2H, J = 7.6), 4.11 (q, 2H, J = 7.1), 4.38−4.41 (m, 1H), 4.60 (d, 1H, J = 15.5), 4.67 (d, 1H, J = 15.5), 5.15 (s, 2H), 5.92 (dd, 1H, J = 15.8, 1.3), 6.26 (t, 1H, J = 7.1), 6.76−6.83 (m, 2H), 7.09−7.42 (m, 7H), 7.84 (d, 1H, J = 7.3), 8.41−8.44 (m, 2H). Anal. ($C_{24}H_{28}N_4O_7$·0.50H_2O) C, H, N.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-Acetylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-phenypropionylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (8).** R_f = 0.12 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3307, 1708, 1666, 1643, 1590, 1519. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.1), 1.70−1.86 (m, 2H), 2.05−2.24 (m, 5H), 3.15 (dd, 1H, J = 13.7, 8.1), 3.50 (dd, 1H, J = 13.7, 7.8), 4.19 (q, 2H, J = 7.1), 4.45−4.56 (m, 1H), 5.66−5.77 (m, 2H), 5.82 (s, 1H), 5.94 (s, 1H), 6.28 (t, 1H, J = 7.2), 6.69 (dd, 1H, J = 15.7, 5.6), 7.10−7.29 (m, 5H), 7.32−7.45 (m, 2H), 8.28−8.36 (m, 2H). Anal. ($C_{25}H_{30}N_4O_6$·0.50H_2O) C, H, N.

***trans*-(2′*S*,4*S*)-6-Carbamoyl-4-{2′-[3″-cyclopentanecarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl]-3′-phenylpropionylamino}hex-2-enoic Acid Ethyl Ester (9).** R_f = 0.27 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3319, 1713, 1666, 1590, 1514. ^1H NMR ($CDCl_3$): δ 1.31 (t, 3H, J = 7.1), 1.55−2.02 (m, 10H), 2.04−2.22 (m, 2H), 2.68−2.80 (m, 1H), 3.16 (dd, 1H, J = 13.7, 7.7), 3.51 (dd, J = 13.7, 8.1), 4.19 (q, 2H, J = 7.1), 4.45−4.56 (m, 1H), 5.57−5.74 (m, 4H), 6.29 (t, 1H, J = 7.4), 6.68 (dd, 1H, J = 15.8, 5.5), 7.10−7.32 (m, 7H), 8.30 (s, 1H), 8.35 (dd, 1H, J = 7.4, 1.7). Anal. ($C_{29}H_{36}N_4O_6$·0.50H_2O) C, H, N.

***trans*-(2*S*,4′*S*)-6-Carbamoyl-4-(2′-{3″-[([1‴,3‴]dithiolane-2‴-carbonyl)amino-2″-oxo-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)hex-2-enoic Acid Ethyl Ester (10).** R_f = 0.28 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3295, 1672 (br), 1590, 1519 (br), 1273. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.1), 1.66−2.22 (m, 4H), 3.17 (dd, 1H, J = 13.7, 7.8), 3.28−3.45 (m, 4H), 3.52 (dd, 1H, J = 13.7, 8.1), 4.19 (q, 2H, J = 7.1), 4.43−4.55 (m, 1H), 5.01 (s, 1H), 5.66 (dd, 1H, J = 15.8, 1.5), 5.67 (s, 1H), 5.86 (s, 2H), 6.29 (t, 1H, J = 7.3), 6.67 (dd, 1H, J = 15.8, 5.5), 7.12−7.40 (m, 7H), 8.31 (dd, 1H, J = 7.3, 1.6), 9.57 (s, 1H). Anal. ($C_{27}H_{32}N_4O_6S_2$·0.50H_2O) C, H, N.

***trans*-(2′*S*,4*S*)-6-Carbamoyl-4-(2′-{2″-oxo-3″-[(tetrahydrofuran-2‴-carbonyl)amino]-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)hex-2-enoic Acid Ethyl Ester (11).** mp 64−67 °C; R_f = 0.28 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3344, 1646, 1519, 1178. ^1H NMR ($CDCl_3$): δ 1.34 (t, 3H, J = 7.2), 1.72−1.82 (m, 1H), 1.95−2.04 (m, 2H), 2.16−2.23 (m, 2H), 2.32−2.43 (m, 1H), 3.18−3.27 (m, 1H), 3.51−3.60 (m, 5H), 3.93−4.00 (m, 1H), 4.05−4.12 (m, 1H), 4.22 (q, 2H, J = 7.2), 4.46−4.55 (m, 2H), 5.54−5.69 (m, 2H), 6.34−6.41 (m, 2H), 6.68 (dd, 1H, J = 15.6, 5.4), 6.86−6.93 (m, 1H), 7.17−7.41 (m, 5H), 8.42−8.45 (m, 1H), 9.37 (d, 1H, J = 10.2). Anal. ($C_{28}H_{34}N_4O_7$·1.5TFA) C, H, N.

***trans*-(2′*S*,4*S*)-6-Carbamoyl-4-{2′-[3″-(2‴,2‴-dimethylpropionylamino)-2″-oxo-2″*H*-pyridin-1″-yl]-3′-phenylpropionylamino}hex-2-enoic Acid Ethyl Ester (12).** R_f = 0.38 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3378, 3307, 3213, 1713, 1666, 1643, 1590, 1514, 1273. ^1H NMR ($CDCl_3$): δ 1.18−1.37 (m, 12H), 1.67−1.98 (m, 2H), 2.05−2.20 (m, 2H), 3.17 (dd, 1H, J = 13.6, 7.7), 3.50 (dd, 1H, J = 13.6, 8.2), 4.19 (q, 2H, J = 7.1), 4.43−4.54 (m, 1H), 5.62−5.72 (m, 2H), 5.81−5.92 (m, 2H), 6.29 (t, 1H, J = 7.2), 6.66 (dd, 1H, J = 15.8, 5.7), 7.13−7.39 (m, 7H), 8.33−8.38 (m, 1H), 8.59 (s, 1H). Anal. ($C_{28}H_{36}N_4O_6$·0.50H_2O) C, H, N.

***trans*-(2′*S*,4*S*)-6-Carbamoyl-4-(2′-{3″-[(5‴-chloroisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)hex-2-enoic Acid Ethyl Ester (14).** mp 163−165 °C; R_f = 0.49 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3335, 1648, 1533, 1179. ^1H NMR ($CDCl_3$): δ 0.34 (t, 3H, J = 7.2), 1.76−1.86 (m, 1H), 1.94−2.00 (m, 1H), 2.23 (t, 2H, J = 6.9), 3.20−3.27 (m, 1H), 3.35−3.42 (m, 2H), 3.51−3.58 (m, 1H), 4.22 (q, 2H, J = 7.2), 4.52−4.57 (m, 1H), 5.69 (dd, 1H, J = 15.6, 1.5), 6.70 (s, br. 1H), 6.14 (s, br. 1H), 6.32 (s, br. 1H), 6.39 (t, 1H, J = 7.2), 6.69 (dd, 1H, J = 15.6, 5.4), 7.21−7.33 (m, 4H), 7.47 (d, 1H, J = 7.2), 8.44 (d, 1H, J = 7.5), 9.47 (s, br. 1H). Anal. ($C_{27}H_{28}ClN_5O_7$·0.60H_2O) C, H, N.

***trans*-(2′*S*,3‴*S*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-phenylpropionylamino]-5-(2‴-oxopyrrolidin-3‴-yl)pent-2-enoic Acid Ethyl Ester (15).** R_f = 0.28 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3272, 1684 (br), 1590, 1514, 1273, 1196. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.1), 1.42−1.55 (m, 1H), 1.59−1.75 (m, 1H), 2.00−2.27 (m, 3H), 3.07−3.28 (m, 3H), 3.43 (dd, 1H, J = 13.7, 7.3), 4.19 (q, 2H, J = 7.1), 4.36−4.47 (m, 1H), 5.12−5.21 (m, 2H), 5.75 (dd, 1H, J = 15.6, 1.2), 5.85−5.94 (m, 1H), 6.26 (t, 1H, J = 7.2), 6.58 (s, 1H), 6.70 (dd, 1H, J = 15.6, 5.7), 7.10−7.41 (m, 10H), 7.44−7.50 (m, 1H), 7.71 (s, 1H), 7.97 (d, 1H J = 6.2), 8.28 (d, 1H, J = 6.8). Anal. ($C_{33}H_{36}N_4O_7$·0.25H_2O) C, H, N.

***trans*-(2′*S*,3‴*R*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-2″-oxo-2″*H*-pyridin-1″-yl)-3′-phenylpropionylamino]-5-(2‴-oxopyrrolidin-3‴-yl)pent-2-enoic Acid Ethyl Ester (16).** R_f = 0.27 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3272, 1684 (br), 1514, 1267, 1196. ^1H NMR ($CDCl_3$): δ 1.31 (t, 3H, J = 7.1), 1.48−1.79 (m, 2H), 2.02−2.24 (m, 2H), 2.27−2.39 (m, 1H), 3.12 (dd, 1H, J = 13.7, 8.2), 3.19−3.34 (m, 2H), 3.48 (dd, 1H, J = 13.7, 7.8), 4.19 (q, 2H, J = 7.1), 4.43−4.53 (m, 1H), 5.17 (s, 2H), 5.73 (dd, 1H, J = 15.6, 1.3), 5.90−5.98 (m, 1H), 6.27 (t, 1H, J = 7.1), 6.63 (dd, 1H, J = 15.6, 6.0), 6.65−6.71 (m, 1H), 7.13−7.27 (m, 6H), 7.31−7.40 (m, 4H), 7.50 (dd, 1H, J = 7.1, 1.6), 7.75 (s, 1H), 7.97 (d, 1H, J = 6.6), 8.69 (d, 1H, J = 7.0). Anal. ($C_{33}H_{36}N_4O_7$·0.50H_2O) C, H, N.

***trans*-(2′*S*,4*S*)-4-[2′-(3″-Benzyloxycarbonylamino-4″-methyl-2″-oxo-2″*H*-pyridin-1″-yl)-3′-phenylpropionylamino]-6-carbamoylhex-2-enoic Acid Ethyl Ester (17).** R_f = 0.23 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3284, 1684 (br), 1596, 1327, (br), 1308, 1267, 1237. ^1H NMR ($CDCl_3$): δ 1.29 (t, 3H, J = 7.1), 1.55−1.68 (m, 1H), 1.82−2.07 (m, 4H), 2.14 (s, 3H), 3.08 (dd, 1H, J = 13.6, 7.1), 3.47 (dd, J = 13.6, 8.8), 4.17 (q, 2H, J = 7.1), 4.41−4.52 (m, 1H), 5.06 (d, 1H, J = 12.3), 5.12 (d, 1H, J = 12.3), 5.59 (dd, 1H, J = 15.7, 1.6), 5.67−5.74 (m, 1H), 5.90 (s, 1H), 6.02 (s, 1H), 6.17 (d, 1H, J = 7.3), 6.68 (dd, 1H, J = 15.7, 5.1), 7.01 (s, 1H), 7.13−7.38 (m, 10H), 7.48 (d, 1H, J = 7.3). Anal. ($C_{32}H_{36}N_4O_7$·0.75H_2O) C, H, N.

***trans*-(2′*S*,4*S*)-6-Carbamoyl-4-(2′-{4″-methyl-3″-[(5‴-methylisoxazole-3‴-carbonyl)amino]-2″-oxo-2″*H*-pyridin-1″-yl}-3′-phenylpropionylamino)hex-2-enoic Acid Ethyl Ester (18).** mp 138−141 °C; R_f = 0.52 (10% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3289, 1663, 1542, 1203. ^1H NMR ($CDCl_3$): δ 1.22 (t, 3H, J = 7.2), 1.62−1.89 (m, 2H), 1.99 (s, 3H), 2.02−2.07 (m, 1H), 2.47 (s, 3H), 3.03−3.39 (m, 5H), 4.12 (q, 2H, J = 7.2), 4.32−4.41 (m, 1H), 5.76 (dd, 1H, J = 15.6, 1.5), 5.80−5.83 (m, 1H), 6.13 (d, 1H, J = 7.5), 6.60 (s, br. 1H), 6.75 (dd, 1H, J = 15.6, 5.4), 7.15−7.24 (m, 5H), 7.76 (d, 1H, J = 7.2), 8.65 (d, 1H, J = 7.8), 9.59 (s, br. 1H). Anal. ($C_{29}H_{33}N_5O_7$·1.5TFA) C, H, N.

***trans*-(2′*S*,3‴″*S*,4*S*)-4-(3′-(4″-Fluorophenyl)-2′-{3‴-[(5‴-methylisoxazole-3‴″-carbonyl)amino]-2‴-oxo-2‴*H*-pyridin-1‴-yl}propionylamino)-5-(2‴″-oxopyrrolidin-3‴″-yl)-pent-2-enoic Acid Ethyl Ester (19).** R_f = 0.24 (5% CH_3OH in CH_2Cl_2). IR (cm^{-1}): 3331, 1690, 1590, 1531, 1455. ^1H NMR ($CDCl_3$): δ 1.30 (t, 3H, J = 7.0), 1.45−1.55 (m, 1H), 1.64−1.75 (m, 1H), 2.03−2.31 (m, 3H), 2.49 (s, 3H), 3.10 (dd, 1H, J = 13.7, 7.9), 3.20−3.46 (m, 3H), 4.20 (q, 2H, J = 7.0), 4.36−4.47 (m, 1H), 5.67 (dd, 1H, J = 15.7, 1.4), 5.85−5.92 (m, 1H), 6.29 (t, 1H, J = 7.2), 6.45 (s, 1H), 6.70 (dd, 1H, J = 15.7, 5.7), 6.86 (s, 1H), 6.90−6.97 (m, 2H), 7.10−7.16 (m, 2H), 7.60 (dd,

1H, J = 7.2, 1.6), 8.37 (dd, 1H, J = 7.2, 1.6), 8.51 (d, 1H, J = 6.6), 9.47 (s, 1H). Anal. ($C_{30}H_{32}FN_5O_7 \cdot 0.50H_2O$) C, H, N.

Oral Bioavailability of Compound 21 in the Beagle Dog. The oral bioavailability of compound **21** was determined after intravenous (15 mg/kg, $n = 3$) and oral (30 mg/kg, $n = 3$) single agent dosing of the molecule to male beagle dogs (BW ~9.3 kg). The compound was administered in a vehicle consisting of 80% propylene glycol and 20% sterile water at a concentration of 15.0 mg/mL. Blood samples were collected periodically for up to 24 h postdosing. The plasma concentrations of **21** were determined by liquid chromatography mass spectrometry (limit of detection equal to 5 ng/mL). Pharmacokinetic parameters were estimated by noncompartmental analysis of the individual plasma concentration−time data.

Plasma Stability Studies. Pooled heparinized human plasma from 10 individuals was obtained from Golden West Biologicals, Temecula, CA, and frozen at −70 °C until analysis. The plasma stability experiment was initiated by transferring 1990 μL of plasma ($n = 3$) or 100 mM potassium phosphate, pH 7.4, buffer ($n = 3$) into separate test tubes. A 10 μL aliquot of a freshly made 5 mM acetonitrile solution of the individual test compounds (**20** and **21**) was transferred into the plasma or buffer to achieve a final compound concentration of 25 μM. The test tubes were incubated at 37 °C for 1−3 h, and 200 μL samples were collected at regular intervals. The samples were mixed with 2 mL of acetonitrile and vortexed to ensure protein precipitation and immediate termination of metabolic transformations. After centrifugation for 10 min at 4000 rpm at 10 °C in a Sorvall RT 7 centrifuge, the clear supernatant was decanted into a new set of tubes and the volatiles were removed under a stream of nitrogen using a Dri-block sample concentrator (Techne, Princeton, NJ). The samples were reconstituted in 250 μL of mobile phase (60% 25 mM, pH 5.1, $NH_4H_2PO_4$ buffer and 40% CH_3CN). Chromatographic analysis was performed using a 1100 Hewlett-Packard high-performance liquid chromatography (HPLC) with a Primesphere reversed phase column (5 μm, 4.6 mm × 15 mm, Phenomenex, Torrance, CA) at a flow rate of 1 mL/min using a gradient elution. A volume of 100 μL was injected onto the column, and the test molecules were detected by UV absorption at 212 nm. The standard curve of the compounds ranged from 0.05 to 40 μg/mL. The half-life of a given compound's metabolic conversion rate was determined by linear regression (WinNonlin Professional, Pharsight, Mountainview, CA) of the mean plasma concentration−time data obtained from the incubation studies.

Microsome Stability Studies. Human pooled liver microsomes (1 mg/mL, consisting of 6−8 livers) and 2 mM NADPH were incubated in 100 mM potassium phosphate buffer, pH 7.4, at 37 °C in a shaking water bath for 1 min. The reaction was initiated by the addition of 25 μM test compound (**19**, **20**, or **21**) and proceeded for 30 min followed by the addition of 500 μL of acetonitrile to terminate the reaction. The incubation volume was 500 μL. Separate control samples were also prepared in a manner similar to the time zero samples with 500 μL of acetonitrile added to the test tube prior to the test compound. All samples were vortexed (2 min) on a SP Multi-tube Vortexer and then centrifuged at 2500g for 20 min. The supernatant was directly injected onto an HPLC system and analyzed. For compound **19**, chromatographic separation was achieved using a Hewlett-Packard 1050 HPLC with a Primesphere C_{18} HC reversed phase column (5 μm, 4.6 mm × 150 mm). Analyte elution was conducted using 25 mM $NH_4H_2PO_4$ buffer, pH 4.5, with constant 5% methanol and a time gradient for acetonitrile of 0−2 min, 5%; 2−10 min, 5−50%; 10−20 min, 50%; 20−21 min, 50−5%; 21−24 min, 5%, with a total run time of 24 min. The retention time of compound **19** was 15.1 min under these conditions. For compounds **20** and **21**, chromatographic separation was achieved using a Zorbax Eclipse XDB C_{18} column (5 μm, 4.6 mm × 150 mm). These compounds were eluted using 25 mM $NH_4H_2PO_4$ buffer, pH 4.5, with constant 10% methanol and a time gradient for acetonitrile of time 0−10 min, 10−55%; 10−16 min, 55−75%; 16−18 min, 75−10%; 18−20 min, 10%, with a total run time of 25 min. The retention times for compounds **20** and **21** were 12.7 and 13.4 min, respectively. All molecules were monitored at 215 nm.

Protein−Ligand Crystal Structure Determination. HRV 3CP of serotype 2 was incubated with a 3-fold molar excess of compound **3** in the presence of 2% dimethyl sulfoxide for 24 h at 4 °C. The resulting complex was concentrated to 7 mg/mL and then passed through a 0.45 μM cellulose−acetate filter. Crystals were grown at 13 °C using the hanging drop vapor diffusion method in which equal volumes (4 μL) of the protein/ligand complex and reservoir solution were layered on plastic coverslips and sealed over wells filled with 1 mL of reservoir solution containing 0.9 M sodium phosphate, 0.9 M potassium phosphate, 0.2 M ammonium sulfate, 0.1 M Hepes at pH 7.5 (HCl titrated), and 20 mM dithiothreitol. A rectangular blocklike crystal of approximate dimensions 0.2 mm × 0.07 mm × 0.07 mm (space group $P2_12_12$; $a = 61.41$, $b = 77.74$, $c = 33.97$ Å) was prepared for low-temperature data collection by transfer to an artificial mother liquor solution consisting of 400 μL of the reservoir solution mixed with 125 μL of glycerol and then flash frozen in a stream of N_2 gas at −170 °C. X-ray diffraction data were collected with a MAR Research 345 mm imaging plate and processed with DENZO.[46] Diffraction data were 96.8% complete to a resolution of 2.3 Å with $R_{sym} = 7.4\%$. Protein atomic coordinates from a previously described isomorphous cocrystal structure of type 2 3CP[15] were used to initiate rigid-body refinement in XPLOR[47] followed by simulated annealing and conjugate gradient protocols. Placement of the inhibitor, addition of ordered solvent, and further refinement were conducted as described previously.[48] The final R factor was 18.4% [(6279 reflections with $F > 2\sigma(F)$]. The root-mean-square deviations from ideal bond lengths and angles were 0.019 Å and 3.2 deg, respectively. The final model consisted of all atoms for residues 1−180 (excluding side chains of residues 12, 45, 55, and 65) plus 141 water molecules.

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